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INTRODUCTION

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IN THIS issue of THE JOURNAL OF PEDIATRICS is presented a series of papers submitted by members of the Department of Pediatrics of the Johns Hopkins University School of Medicine, who are members of the staff of the Harriet Lane Home and of the Johns Hopkins Hospital or of the Pediatric Services of the Sinai Hospital under Dr. Harry Gordon or of the Baltimore City Hospitals under Dr. Harold Harrison. These papers were selected to represent certain important areas within pediatrics.

Darrow pointed out in his presidential address at the 1957 meeting of the American Pediatric Society that the clinical departments of medical schools, just as other departments of a university, are concerned with the *advancement* of knowledge and not just the *preservation* and *transmission* of knowledge. Departments of pediatrics are presently organized to study not only isolated diseases which affect infants and children more frequently than adults but also the increasingly important area of human biology which is compressed into the period from birth through adolescence. Over this time, profound changes of a qualitative and quantitive nature occur which offer unlimited opportunities for fundamental research.

Departments of pediatrics had their beginnings in most medical schools as offsprings of departments of medicine or of women's diseases. These departments were created largely for the practical training of individuals in this specialty and this mission has been carried out very effectively over the last 30 years. The role of a department which is restricted to the training of people for the practice of a specialty limits the importance of such a department and is not consistent with the definition of a university department alluded to above. University departments of pediatrics at the present time must be devoted largely to research in human biology rather than the practical training of individuals, although this may be an important by-product of the activities of the department.

The role of pediatrics in the medical curriculum has also changed over the past 50 years. First, there was solely a short series of lectures; then, in addition to the lectures, there was clinical participation as an elective in ward work; then more and more schools included required clerkships in the third

year or fourth year, and most schools in both years. The reason behind this expansion in most instances was a very practical one. At least one third of the average practitioner's day was found to be spent in the care of children. Completely apart from these practical considerations, pediatrics should become of increasing importance in medical education. Although pediatrics is an outgrowth of medicine and obstetrics, in actual fact the generality, not the specialty, of pediatrics is the reason why pediatrics will become of increasing importance in the medical education of the future. This field encompasses a wider scope than any other clinical activity. In physical terms patients are cared for from the 1-pound premature infant to the 260-pound adolescent. In psychologic terms the range is from the totally disorganized infant with hydranencephaly to the individual with superior intellect entering graduate school at 14. Pediatrics is concerned with problems of growth, maturation and learning, nutrition, infection, trauma, hypersensitivity, neoplasia, even occasionally premature senility. Pediatrics thus is the broadest area of clinical medicine. Natural selection, except for failure of conception or abortion or miscarriage, has not yet operated. We turn over to our colleagues in adult medicine a selected population. Actually, in this sense, adult medicine is a specialty. For example, of what importance is mental deficiency to the internist, despite the fact that 5 per cent or so of our population is so affected? What is the significance of disturbances of growth or disturbances of behavior? Only recently has interest in congenital anomalies been engendered in adult medicine. Of even greater importance is the fact that in order to prevent disabling disease in the adult, the genesis of such disease in the formative years of life must be studied. It appears, therefore, that a solid foundation of pediatrics would be essential for the internist and psychiatrist. For even the uninitiated it should seem obvious that personality development is an intimate part of pediatrics and that much understanding can come from the anterospective study of infants and children rather than from a total retrospective approach. An example of note is the study by Money and the Hampsons of cases of the adrenogenital syndrome. With these patients, they demonstrated the importance of early parental attitudes in determining adult psychosexual orientation. In regard to the so-called degenerative diseases there is considerable evidence that factors such as obesity, hyperexcitability, etc., contribute to the development of cardiovascular disease. Likewise, eating patterns and other modes of reaction are established in the pediatric period so that the eventual solution to certain degenerative disorders would seem to me to depend upon preventive adult medicine operating during childhood.

This broad base of clinical material is not all that pediatrics has to offer. Pediatrics is as close to the basic sciences as any other clinical subject. Nowhere in medicine is the importance of embryology, biochemistry, genetics, nutrition, immunology, and psychology more obvious to the student than in pediatrics. Here indeed is "disease in pure culture." The study of congenital heart disease brings home to the student the obvious need for review of embryology of the cardiovascular

system, as do the multiple genitourinary anomalies and those of the gastrointestinal system. The recent discovery of specific enzymatic disorders, such as galactosemia, highlights for the student the intricacies of intermediary carbohydrate metabolism. The findings of Wilkins and others in pediatric endocrinology are unforgettable examples and serve as illustrations to the student of the details of steroid metabolism. The chromatin abnormalities in the Mongolian infant point out in dramatic fashion the importance of fundamental biology in the clinic as well as the laboratory.

The charge may be levied that each department of pediatrics might become in itself a small medical school. Obviously a compromise must be made between sources of funds, space, personnel, etc. The development of an area of investigation such as hematology in a pediatrics department should not be handicapped because there is the presence of a good division in adult medicine. If this were the case, research in child care would return to the same state in which it was in 1909. Pediatric hematology is concerned with maturational problems as well as a number of congenital problems and with nutritional problems such as iron metabolism and others and can stand quite independently on its own accomplishments and justifies its own support.

Universities must also supply leadership in the field of pediatric practice. The public is demanding that pediatricians care for all of their children. By contrast, some academic pediatricians are raising doubts as to the need for the practicing pediatrician and are suggesting the return of the well-trained family physician who turns over difficult problems to the academic pediatrician. I do not believe that such generalization of care will return. First, the public will not permit such a step. More important, however, such a physician, in the era of increasing complexity of medicine, might become little more than a good visiting nurse; his own interest in new advances and his index of suspicion would soon become blunted. The analogy to the "old family physician" is inappropriate. Vast knowledge is accumulating in many fields for which he must be responsible. The family doctor of years ago had to be thoroughly familiar with all phases of medical practice since few specialists were available. His interest remained high because he was not a general practitioner but a general practitioner *and* specialist. Such a situation does not exist now. Instead, to guarantee excellence of care, especially from a preventive standpoint, specific knowledge of the practicing pediatrician must be increased and opportunity for intensive postgraduate study must be developed. To permit continuity of care, the two periods of transition—prenatal to natal and adolescence to adulthood—must be covered by joint participation of appropriate specialists.

It is the purpose of this Hopkins issue to point out certain areas which are considered of importance in a moderately large department of pediatrics. The work that is selected in this JOURNAL does not represent all the research activities of the department or necessarily those which might be considered the most fundamental. Selection is based upon a representation of areas to give a more rounded picture of what modern departments

are concerned with. The various topics which are presented in no way reflect the relative importance of problems within pediatrics. All represent important aspects of human biology.

The paper by Odell represents interest in the physiology of the newborn infant and illustrates the importance of certain fundamental physical chemical principles in the prevention of brain damage. The studies of Dr. Hardy illustrate the preventive aspects of pediatrics from a standpoint of earlier detection of hearing deficits, with emphasis on earlier treatment if earlier detection is possible. The review by Chisolm demonstrates the importance of biochemical procedures in diagnosis and the need for increasing awareness of the importance of amino-acid metabolism in diseases of children. Dr. Gordon's manuscript emphasizes that all is not dead in the nutritional aspects of pediatrics, with conclusive evidence of the role of vitamin E as an essential factor in human nutrition. The paper of Migeon points out clearly the developmental aspects of steroid metabolism. That of Gamble shows an aspect of the handling of water and electrolytes by the body that has been studied almost entirely by pediatricians. The paper of Nyhan on the intermediary metabolism of tumor tissue brings to the clinician a rational approach to the development of anticancer agents. These studies demonstrate the need for complete familiarity with the basic problems in biochemistry if clinical problems are to be solved. This work is included because of the growing importance of the field of neoplasia in children.

No department of pediatrics can be considered complete without considerable interest in the area of genetics. The paper presented by Klein and Sidbury emphasizes anatomical features of genetic disorders which can be detected only by the use of sensitive laboratory tools such as the skin resistance method developed by Richter. Equally good examples of the heterozygous state or partial expression can be found in the use of biochemical approaches. The work described by Zinkham is illustrative of the modern approach to hematology which is not concerned with morphology alone but rather with actual analysis of the enzymatic components of blood cells controlling survival and function and their correlation with genetic factors and other environmental influences.

Medearis' work is illustrative of the role and importance of infectious disease in pediatrics. Although cases of infection may not constitute as large a part of the patients hospitalized in many areas of the country as formerly, at Harriet Lane this still constitutes a major problem. The study of infection along with that of deviations in resistance will continue to be of great importance in the study of the fetus, newborn infant, and infant. With the great advances in pediatric cardiology, most departments of pediatrics have active groups engaging in the study of problems in cardiology; the paper of Neill and Taussig is illustrative of this important aspect.

Behavioral pediatrics is not new. Interest in the development of personality and in abilities to learn dates back to the early periods of child study. The paper by Mark illustrates the contributions that may be made by a psychologist who is confronted with abnormalities in information processing. The work on communication disorders

is an example of the application of experimental psychology to clinical problems and there will be increasing interest in this area. The review by Dr. Leo Kanner closes the issue from this department and quite appropriately emphasizes the many factors that go into the complex emotional make-up of the child as he develops into an adult. It is particularly appropriate that Dr. Kanner has made this contribution at the time of his official retirement.

It is hoped that other departments will take the opportunity to collect some of their work in such issues to present to the practicing physician the vast expanse of problems in child health and disease under study in the many universities throughout the world. It is also hoped that the practicing physician and pediatrician will gain respect for the activities of departments of pediatrics everywhere by such presentations of research interests and contributions, and that interest will be encouraged to the point of participation and support of the activities of these departments.

THE DISSOCIATION OF BILIRUBIN FROM ALBUMIN AND ITS CLINICAL IMPLICATIONS

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THE association of hyperbilirubinemia in the newborn period and in kernicterus is well known. A causal relationship between unconjugated (indirect) bilirubin and kernicterus, although not completely proved, is probable. A number of in vitro studies have demonstrated the cytotoxicity of bilirubin to such fundamental cellular processes as cell respiration,[1] oxidative phosphorylation,[2] and electron transport.[3] The toxic concentration of bilirubin in these in vitro studies was about 20 mg. per cent, a plasma concentration associated at times with kernicterus in newborn infants. However, the in vitro studies were done with isolated cells or cell fragments, and therefore doubt may exist as to how applicable the results of these in vitro studies are to the in vivo toxicity of bilirubin. Since kernicterus has been observed in infants with serum concentrations considerably below 20 mg. per cent and concentrations in excess of 20 mg. per cent have been innocuous, it is apparent that factors other than plasma concentration are of importance.

The factors controlling distribution of bilirubin in the body are only partially understood. A number of studies[4-9] have shown that the plasma bilirubin concentration does not necessarily indicate how much bilirubin may be present in extravascular tissues. Consequently, plasma bilirubin concentrations may be only a rough guide as to the bilirubin concentration of tissues. Recent studies from this laboratory on bilirubin distribution in the body have emphasized the need for a distinction between total serum bilirubin concentration and that fraction of the total which is diffusible.[10] Martin's studies[11] have shown that at pH 7.4 the maximal concentration of bilirubin in aqueous solutions is 0.1 mg. per cent, and therefore in icteric serum practically all of the bilirubin is bound. If we presume that in order for bilirubin to enter cells it must dissociate from its bound state, the diffusible (dissociated) bilirubin concentration is of a more fundamental importance than total bilirubin concentration. The dissociated fraction of the total circulating bilirubin will determine the diffusion gradient across cell membranes. The direction of the gradient at any one moment will be a function of the concentrations of dissociated

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bilirubin on each side of the cell membrane rather than the total amounts of bilirubin on either side (Fig. 1).

The state of unconjugated bilirubin in serum has been studied by many investigators[10-16] by a number of techniques. In conditions of hyperbilirubinemia the bulk of the circulating bilirubin is attached to serum albumin. There is reason to believe that bilirubin is bound to the albumin in a manner similar to the albumin binding of many other organic anions. Furthermore, the binding of these anions can be expressed stoichiometrically in terms of the chemical laws of mass action by the general equation[17]

$$(1)\quad \frac{[Alb^{+}][B^{-}]}{[Alb\text{-}B]} = K,$$

where Alb and B represent albumin and the anion, respectively, and K is a dissociation constant of the albumin-anion complex. From Martin's studies[11] it is known that 1 mole of albumin will bind almost 2 moles of bilirubin at pH 7.4. Therefore, the albumin bilirubin binding reaction may be described by equations 2 and 3.

$$(2)\quad \frac{[Alb^{++}][B^{-}]}{[^{+}Alb\text{-}B]} = K_1$$

$$(3)\quad \frac{[^{+}Alb\text{-}B][B^{-}]}{[B\text{-}Alb\text{-}B]} = K_2$$

It has been shown[17] that if 1 mole of albumin binds 2 moles of organic anion with the same intrinsic affinity at each site of binding, the dissociation constant (K_2, equation 3) describing the dissociation of the second mole of anion from the albumin will be four times greater than the dissociation constant describing the dissociation of the first mole of anion (K_1 of equation 2).

Therefore, albumin binds 1 mole of bilirubin more tightly than 2 moles. The specific molecular sites of bilirubin and albumin responsible for the complex formation are not definitely known but probably involve the cationic amino groups of albumin and the carboxyl groups of the propionic acid side chain of bilirubin.

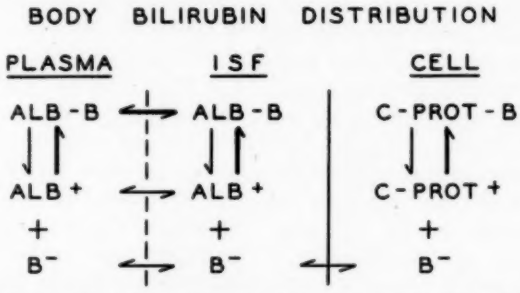

Fig. 1.—Schematic representation of the chemical state of bilirubin and its distribution in the body between the plasma, interstitial (ISF), and cell compartments. The dashed vertical line represents vascular endothelium through which albumin is permeable. The solid vertical line represents the cell wall which is impermeable to albumin. C-Prot-B represents a cell protein-bilirubin complex. Alb-B represents the bilirubin-albumin complex. The dissociated bilirubin (B^-) will diffuse into the various compartments depending upon the dissociated bilirubin concentration in the respective compartments. At equilibrium the absolute concentration of free bilirubin (B^-) in the ISF will be less than in the plasma because there is considerably less protein. Consequently, the dissociated bilirubin concentration in plasma or, ultimately, the saturation of the plasma proteins with bilirubin determines whether or not bilirubin diffuses into or away from interstitial fluid.

It is also not known whether albumin binds both moles of bilirubin with the same intrinsic affinity or if the bilirubin already bound interferes with the binding of a second mole by electrostatic as well as statistical factors, which would make the dissociation constant $K_2 > 4K_1$.

One of the implications from equations 2 and 3 is that alteration of the serum albumin concentration will also affect the total and dissociated bilirubin concentrations. If the serum albumin concentration is low as in premature babies,[18] the albumin becomes saturated with bilirubin at lower bilirubin concentrations. Any newly formed bilirubin remains either dissociated and readily diffusible to extravascular tissues or weakly attached on other plasma proteins.[13] In either event the concentration of diffusible bilirubin would be relatively high and the likelihood of kernicterus enhanced.

A means of reducing the binding capacity of serum for bilirubin without reducing total albumin concentration is to introduce other organic anions which are bound by albumin at the same locus as bilirubin or alter the ability of this locus to bind bilirubin. The presence of such organic anions would compete with bilirubin for binding and reduce the amount of bilirubin which is protein bound and thereby increase the dissociated (diffusible) bilirubin. The clinical observations of Silverman and coworkers[19] are pertinent in this respect. Silverman and his associates noted that premature infants treated with the sulfonamide, sulfisoxazole, developed kernicterus six times more frequently than comparable control infants. Yet the serum bilirubin concentrations were higher in the unaffected control infants.[20] Johnson[21] has observed a similar phenomenon in the congenitally icteric Gunn-strain rat. The incidence of kernicterus in the litters from adult female rats was increased by administration of sulfisoxazole to the newborn animals, yet the serum bilirubin levels were lower in the affected animals than in the nontreated controls. We have found sulfisoxazole will displace albumin-bound bilirubin in vitro, allowing the ultrafiltration or dialysis of bilirubin through protein-impermeable membranes.[22] Consequently, it is reasonable to believe that experimental animals or premature infants when given sulfisoxazole had lower serum bilirubin levels not because it was excreted but rather because the sulfonamide prevented bilirubin from attaching to serum proteins, thus promoting its diffusion into tissues. The long-acting sulfonamide sulfamethoxypyrimidine has been found to be more effective than sulfisoxazole in displacing albumin-bound bilirubin. Salicylate is more effective than either of the sulfonamides in this type of reaction on a weight as well as a molar basis.

In Fig. 2 is illustrated the relative effectiveness of these drugs in displacing protein-bound bilirubin. The technical details for obtaining these data are presented elsewhere,[10] but the essentials of the method for such experiments are as follows:

The patient's serum was diluted 50 fold in isotonic phosphate buffer pH 7.4 and 2.5 ml. placed in 4 cuvettes. The spectral absorption curves of the samples were determined in a Beckman DU spectrophotometer between

400 and 500 mμ using water as a blank. Subsequently, to the control cuvette sample 20 λ volumes of buffer were added. To the other 3 cuvette samples, 20 λ volumes of 400 mg. per cent solutions of sulfisoxazole, sulfamethoxypyrimidine, and sodium salicylate were added, respectively. After each addition, the optical density was measured at 465 mμ, the absorption maximum of protein-bound bilirubin. The loss of optical density at 465 mμ in the experimental cuvettes was compared to the loss in the control cuvette and the difference in loss expressed as per cent change in optical density (% Δ O.D. 465 mμ). This is a minimal measure of the amount of bilirubin dissociated from serum protein.

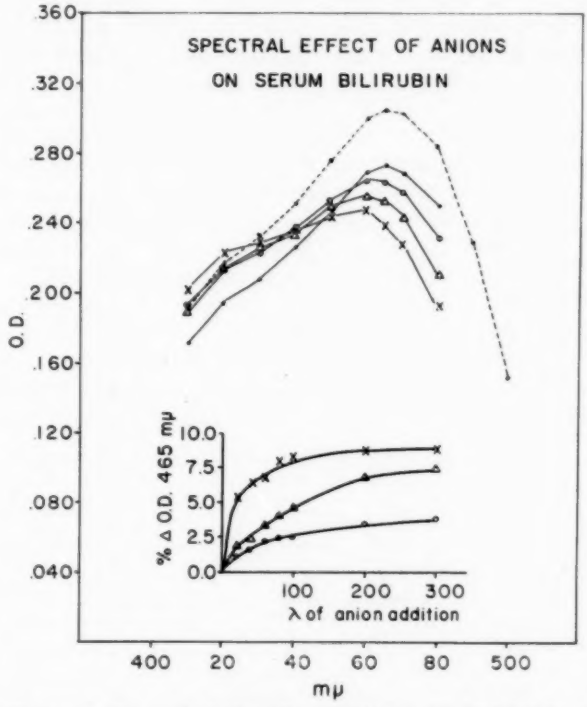

Fig. 2.—Changes in the spectral absorption of icteric serum induced by organic anions.

The serum from an erythroblastotic infant with a bilirubin concentration of 0.8/21.2 mg. per cent was diluted 50 fold with isotonic phosphate buffer pH 7.4 and 2.5 ml. placed in 4 cuvettes. The anion solutions were added in 20 λ aliquots to the appropriate cuvette. All of the anion solutions added were of a 400 mg. per cent concentration. The insert illustrates the per cent of the total protein-bound bilirubin dissociated (% Δ O.D. 465 mμ) plotted against each volume of anion solution added. The spectral curves above the insert are of the samples before addition and after the final addition of either buffer or anion solution had been made.

●- - -●, control and experimental cuvette samples before additions.
●——●, control sample after 300 λ addition of buffer.
○——○, sample after 300 λ of sulfisoxazole solution was added.
Δ——Δ, sample after 300 λ of sulfamethoxypyrimidine was added.
×——×, sample after 300 λ of sodium salicylate was added.

The effect of each addition is plotted in the insert of Fig. 2. It is apparent that salicylate caused the greatest dissociation of bilirubin from protein, and sulfisoxazole the least.

After a total of 0.3 ml. of buffer or anion solution had been added to the appropriate cuvette samples, the spectral absorption curves were determined from 410 to 480 mμ and are shown in Fig. 2. It can be seen that the shape of the curve for the control sample remains unchanged and the lower absorption values are due to simple dilution by the added buffer. However, the cuvette samples containing the added organic anion are quite different. The greater loss of optical absorption at 465 mμ was accompanied by a rise in absorption at 420 mμ. The 420 mμ peak is characteristic of solutions containing free bilirubin. Parenthetically, it might be added that this type of methodology is useful in testing chemical agents for their effect on protein-bound materials.

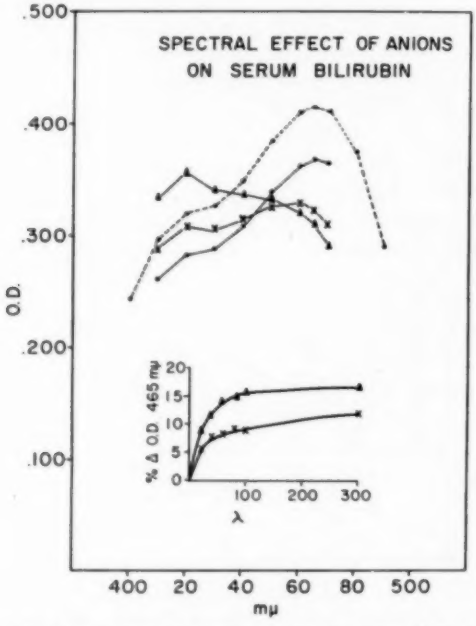

Fig. 3.—Changes in the spectral absorption of icteric serum induced by organic anions.

The serum from an erythroblastotic infant with a bilirubin concentration of 1.0/26.0 mg. per cent was prepared as described in Fig. 2. The anion solutions employed were a 4 mg. per milliliter sodium salicylate solution and a 64 mg. per milliliter solution of caffeine sodium benzoate.

The insert below illustrates the per cent of the total protein-bound bilirubin dissociated (% Δ O.D. 465 mμ) plotted against the volume of anion solution added to the 2.5 ml. cuvette samples of diluted serum.

The spectral curves above are of the cuvette samples before additions and after the final addition of either the buffer or anion solution had been made.

●- - -●, control and experimental cuvette samples before any additions.

●——●, control sample after 300 λ of buffer was added.

×——×, sample after 300 λ of sodium salicylate was added.

▲——▲, sample after 300 λ of caffeine sodium benzoate was added.

Caffeine sodium benzoate is another drug frequently employed in the management of premature infants. This material in solution is really a mixture of caffeine and sodium benzoate. Such solutions have

long been known to affect protein binding of bilirubin[23] by causing the detachment of bilirubin from protein, allowing it to filter through membranes impermeable to protein. The effect of caffeine sodium benzoate solutions on protein-bound bilirubin of serum is shown in Fig. 3 and compared with the effect of salicylate. The results used for Fig. 3 were obtained by the technique outlined above for Fig. 2. The serum was from an erythroblastotic infant, and the caffeine sodium benzoate used was the solution employed as a respiratory stimulant in the premature nursery, containing 64 mg. per cubic centimeter of caffeine sodium benzoate. It is apparent that the caffeine solution behaves qualitatively like the salicylate solution in reducing optical density at 465 mμ, the absorption maximum for protein-bound bilirubin. The loss at 465 mμ is accompanied by a concomitant rise at 420 mμ, the absorption maximum for free bilirubin. We do not know whether this effect is due to the benzoate ion competing with bilirubin for its binding locus or whether there is some sort of caffeine bilirubin complex formation such as occurs between caffeine and heme[24] or both. The detachment of bilirubin from protein may explain why the use of caffeine sodium benzoate accelerates the indirect reacting fraction of serum bilirubin in the van den Bergh reaction.[25] With[26] has shown that bilirubin solutions containing caffeine behave differently in the diazo reaction. The azo-pigment products have a different absorption spectrum than the azo-pigments from the van den Bergh reaction in the absence of caffeine sodium benzoate. It is not possible to say from the above observations whether the clinical use of caffeine sodium benzoate results in a greater incidence of kernicterus, but testing for this effect in the experimental animal seems indicated.

TABLE I. THE INFLUENCE OF ORGANIC ANIONS ON CHLOROFORM EXTRACTION OF BILIRUBIN FROM SERUM

TUBE SAMPLE		ADDITION TO AQUEOUS LAYER	CHLOROFORM LAYER	
			ε O.D. 450 Mμ	BILIRUBIN CONCENTRATION μG/ML.
Control	1	0.1 ml. of isotonic PO_4 buffer	0.234	2.47
	2	0.1 ml. of isotonic PO_4 buffer	0.238	2.50
	3	0.1 ml. of 20 mg. per cent hematin	0.250	2.63
	4	0.1 ml. of 20 mg. per cent hematin	0.251	2.64
	5	0.1 ml. of caffeine sodium benzoate, 64 mg. per milliliter	0.263	2.77
	6	0.1 ml. of caffeine sodium benzoate, 64 mg. per milliliter	0.262	2.75
	7	0.2 ml. of caffeine sodium benzoate, 64 mg. per milliliter	0.275	2.89

The serum before dilution contained a bilirubin concentration of 1.6/29.6 mg. per cent.

In hemolytic anemias of the newborn infant neurologic signs of kernicterus may be seen when plasma concentrations are relatively low.[27, 28] In these anemias the levels of circulating heme pigments may be elevated. It has been suggested that competition between the heme pigments and the bilirubin exists for a common binding locus on the albumin molecule. If such were true, it

is possible that the diffusible bilirubin concentration may be elevated despite relatively low total serum bilirubin concentrations. It has been found in this laboratory that salicylate and sulfonamides will detach hematin bound to albumin as methemalbumin, shifting the spectral absorption maximum from the protein-bound peak of 405 mμ to a lower value of from 380 to 390 mμ, the absorption maximum of free hematin. By adding crystalline bovine hematin to icteric human sera from jaundiced infants, one can subsequently extract more bilirubin from the serum. Table I shows the results of such a procedure.

The patient's serum was diluted 50 fold with isotonic phosphate buffer, pH 7.4. Two milliliter aliquots were placed in centrifuge tubes and to each tube 2 ml. of chloroform was added. To the duplicate control tubes, 0.1 ml. of buffer was added and to the experimental tubes was added 0.1 ml. of 20 mg. per cent crystalline bovine hematin in N/20 NaOH. The tubes were shaken to permit mixing of the aqueous and chloroform phases and subsequently allowed to stand 30 minutes in the icebox for separation of the liquid phases. The lower chloroform layer was then analyzed for bilirubin concentration spectrophotometrically.

From the table it is apparent that the tubes to which hematin was added yielded more bilirubin in the chloroform layer, which would only occur if hematin interfered with the protein binding of bilirubin. A similar result is obtained when, instead of human sera, a model serum is similarly treated. The model serum consists only of a mixture of a crystalline bilirubin solution and crystalline bovine albumin in a phosphate buffer of pH 7.4. One also finds that addition of caffeine sodium benzoate, salicylate, or sulfonamides to serum increases the amount of bilirubin extractable by chloroform. It therefore appears possible that hematin, in vivo, as methemalbumin may effectively reduce the albumin-binding capacity of the serum. As a consequence, dangerous levels of diffusible bilirubin may occur when total serum bilirubin concentrations are relatively low. Such a conception is compatible with the view of Abelson and Boggs[27] and Tuttle[28] that exchange transfusion is indicated when the circulating heme pigments are elevated as indicated by the spectral absorption of serum in the 405 to 415 mμ range. Their technique has the unfortunate disadvantage that any hemolysis occurring due to the technique of collection of the blood samples interferes with the reliability of the spectral absorption measurements for heme pigments.

Another implication from equations 2 and 3 is that elevation of the serum albumin concentration should effectively raise the concentration of bilirubin which serum could sustain before saturation is reached. Also, by reference to Fig. 1 and equations 2 and 3, it can be seen that the administration of albumin would reduce the concentration of diffusible bilirubin in plasma. Such an effect would promote a shift of dissociated bilirubin from extravascular tissues toward the plasma to reach equilibrium again between the concentrations of dissociated bilirubin in the fluid phases of the body. The new equilibrium state could only be reached by bilirubin dissociating from its bound state in tissues to replace the free bilirubin lost

by diffusion into the plasma. Recently we have tested this postulate by administering salt-poor concentrated albumin to jaundiced infants (5 to date). An example of the uniform response we have thus far observed is shown in Table II and in the following case description.

respectively. Thus, the serum protein concentration rose only 0.3 Gm. per cent, illustrating the large volume of distribution of serum albumin in the infant. The maximum rise of the indirect-reacting bilirubin during this interval was 4.4 mg. per cent. The subsequent fall of the baby's serum bilirubin concentration was presumably due to excretion, for he

TABLE II. THE EFFECT OF INTRAVENOUSLY ADMINISTERED ALBUMIN ON THE SERUM BILIRUBIN CONCENTRATION

DATE	TIME	BILIRUBIN CONCENTRATION (MG. %)			TOTAL SERUM PROTEIN CONCENTRATION (MG. %)
		DIRECT	TOTAL	INDIRECT	
2/25	4:00 P.M.	1.0	18.4	17.4	
	10:00 P.M.	(not done)	22.0		
2/26	9:00 A.M.	1.0	18.8	17.8	
	3:00 P.M.*	2.2	16.8	14.6	5.0
	3:45 P.M.	1.8	19.2	17.4	
	5:00 P.M.	1.4	20.4	19.0	5.3
	11:00 P.M.	1.0	20.0	19.0	
2/27	9:00 A.M.	1.4	17.6	16.2	
2/28	9:00 A.M.	2.0	10.0	8.0	

*Salt-poor albumin, 2.25 Gm., was given intravenously as 9 c.c. of a 25 per cent solution.

CASE REPORT

The infant was a 1,700 gram premature infant who had hyperbilirubinemia for which excessive hemolysis could not be demonstrated as a cause. Nine cubic centimeters of 25 per cent salt-poor albumin (2.25 Gm.) was given intravenously, as indicated in Table II, on the fifth day of life when the serum bilirubin concentration was falling. Any rise in bilirubin concentration occurring after the albumin administration could thus be interpreted as bilirubin shifted from extravascular tissues into the plasma presumably attached to the administered albumin. As illustrated, the indirect bilirubin had fallen to 14.6 mg. per cent at the time of albumin administration. Forty-five minutes later the bilirubin was 17.4 mg. per cent, and at 120 minutes after albumin administration the bilirubin concentration was 19.0 mg. per cent. The total protein concentration of the serum before and 2 hours after the albumin administration was 5.0 and 5.3 Gm. per cent, became progressively less icteric. It cannot be interpreted that the albumin increased the rate of excretion because the albumin was deliberately given at a time when the infant would normally be expected to have diminishing icterus.

It may be that the extra bilirubin, which appeared in the plasma from extravascular tissues, was derived largely from the liver. At worst, this may actually reduce the rate of excretion of bilirubin for it would reduce the amount of free bilirubin available for excretion. At best, if the extra bilirubin came from tissues other than liver, the administered albumin may have promoted excretion for it increased the total delivery of bilirubin for excretion to the organ responsible for this function. Also, it may be criticized that increasing the circulating bilirubin may increase the amount passing by brain capillaries and blood-cerebrospinal fluid

barriers. However, at equilibrium, the net effect of albumin as discussed above can only be to reduce total tissue bilirubin by its common ion effect in a multiple equilibrium system involving free and bound bilirubin.* Also, it should be recalled that in the clinical experience of Silverman and colleagues[19] the plasma bilirubin levels were lower in the group of infants who developed kernicterus. The relative rapidity with which the serum bilirubin rose after albumin administration may have therapeutic implications. If an infant is "primed" with albumin before exchange transfusion, more bilirubin may be removed without the need for prolonging the time of exchange transfusion. Also, the use of albumin may be more effective than whole serum as shown by Blondheim's studies discussed below. The response of the serum bilirubin to the albumin administration may explain the different "rebound" behavior of serum bilirubin when exchange transfusions are done with whole blood[5] versus packed cells.[20]

By extrapolation of the 4.4 mg. per cent rise in serum unconjugated bilirubin associated with the 0.3 mg. per cent rise in serum albumin, a 1 Gm. per cent rise of serum albumin could raise the serum unconjugated bilirubin 14.6 mg. per cent. This rise is close to Martin's value[11] which is that 1 Gm. of albumin could bind 15 mg. of bilirubin in vitro at pH 7.4.

It should be kept in mind that Martin's study was done with crystalline human albumin and bilirubin in an aqueous phosphate buffer. In such a system there were no other anions competing with bilirubin for protein binding; consequently, all of the albumin could participate in the formation of the albumin bilirubin complex. Blondheim's studies[30] have shown that this is not true for the albumin in whole sera. The latter investigator has shown, when using phenolsulfonphthalein (PSP) as the test organic anion, that the first 1.5 Gm. per cent of albumin in whole sera did not participate in the formation of the anion protein complex. However, when using crystalline albumin alone, all of the albumin participated in binding the PSP. If these results can be applied to newborn infants' serum, only 1.5 Gm. per cent out of the total 3 Gm. per cent serum albumin is available for binding bilirubin. This would reduce the infants' serum bilirubin saturation level to about 22 mg. per cent which is the level considered so dangerous to the neonate. Furthermore, this amount of binding is for a pH of 7.4 which is higher than the usual venous or capillary pH of newborn infants. Martin[11] has shown a marked pH sensitivity of the albumin binding of bilirubin. At pH 7.9, 1 mole of albumin bound 3.3 moles, while at pH 7.4, 1 mole of albumin bound only 1.9 moles of bilirubin. Electrophoresis studies have shown bilirubin dissociates completely from protein[16] at pH 7.0 and below. It would therefore appear that pH is another important variable in determining the binding of bilirubin and consequently its diffusion.

*This statement applies only at equilibrium. However, if disequilibrium should occur such as a sudden local fall of pH within the brain cells, the free bilirubin concentration within the cells would be reduced by precipitation. It is then possible that a high concentration of circulating bilirubin would allow a larger absolute amount of bilirubin to diffuse across the cell membranes even though the saturation of the plasma was no greater than at lower plasma bilirubin concentrations.

The distribution of bilirubin in the body might be considered from a viewpoint that many tissues bind bilirubin. Each tissue fraction would have its own binding affinity for bilirubin expressed in terms of a dissociation constant. The relative values of these dissociation constants and the amount of bilirubin available for diffusion will determine the direction of distribution of the bilirubin in the body as a whole. It would appear that the K_1 in equation 2 of the bilirubin-albumin complex is considerably less than the dissociation K of other tissue-bilirubin complexes but not the K_2 of equation 3. This may explain why infants are not visibly jaundiced at birth and yet may have high cord serum bilirubin levels. In utero the placental circulation provides abundant maternal albumin to direct the diffusion gradient of bilirubin of fetal origin away from the infant.

The effect of membrane permeability has not been included in this discussion, but quite obviously it can influence the rate of diffusion but not the equilibrium of binding between bilirubin and tissue proteins of the cells.

The reported experiments[31-33] of infusion or injection of bilirubin indicate a difference in permeability to bilirubin in young versus more mature animals of the phase boundaries separating the hemocirculation from the central nervous system. However, it is impossible to know whether the circulating plasma proteins were as saturated with bilirubin in the perfusions of the young versus the mature animal so that comparable concentrations of diffusible bilirubin were obtained. In view of the lipid and water solubility of bilirubin it appears reasonable to consider the difference of blood-brain barrier permeability to bilirubin in the young versus the mature animal as a difference in rates of diffusion as discussed by Bakay[34] rather than as an all-or-none phenomenon. The experimental as well as clinical evidence suggests that maturation of the blood-brain barrier may be a vital factor in the pathogenesis of kernicterus. Recent reports[8, 9, 35] of the concentrations of bilirubin in cerebrospinal fluid (C.S.F.) of infants have suggested that this may be a useful parameter in deciding the need for exchange transfusion because the concentration of bilirubin may indicate the relative maturation of the blood-brain barrier. However, it remains to be proved that bilirubin of the C.S.F. is able to diffuse into the brain substance and cause kernicterus. The C.S.F. bilirubin may well represent bilirubin dialyzed from plasma across the blood-cerebrospinal fluid barrier because of the presence of plasma proteins present in C.S.F. from birth before hyperbilirubinemia occurred. The studies of C.S.F. bilirubin[8, 9, 35] certainly show a close relationship between the concentration of bilirubin and plasma protein in the C.S.F. If, therefore, this bilirubin is bound to protein, it may be quite innocuous. From the anatomical pathology of kernicterus, it seems more likely that the bilirubin which is toxic gains access to the brain via the cerebral capillary vessels. Therefore, the diffusible bilirubin concentration of the cerebral plasma circulation may be the more important variable in determining the likelihood of kernicterus.

SUMMARY

The distribution of bilirubin in the body has been described in terms of diffusion equilibria and competitive binding of tissue elements for the circulating bilirubin which is chemically dissociated from albumin. The supposition was made that only dissociated bilirubin can gain access to intracellular fluid by permeation of cell membranes. The amount of bilirubin diffusing into cell fluid is then dependent upon the concentration gradient of dissociated bilirubin across cell membranes rather than total bilirubin concentration.

It was shown that the dissociated bilirubin concentration can be increased in extracellular fluids independently of total bilirubin concentration by reducing the binding capacity of plasma proteins for bilirubin. This situation occurs clinically (1) in premature babies who have a relative hypoalbuminemia, and (2) in some newborn infants whose plasma contains substances which reduce the bilirubin binding capacity of albumin. Such substances are hematin, sulfonamides, salicylate, caffeine sodium benzoate, and increased hydrogen ion concentration.

By increasing the circulating albumin concentration, it was shown that the administered albumin will increase the total plasma bilirubin concentration by causing a shift of bilirubin from the extravascular tissues into the plasma. The potential therapeutic value of such a procedure was discussed but requires further study before it can be recommended.

I wish to acknowledge my indebtedness to Dr. H. H. Gordon and Dr. R. E. Cooke for their interest in and suggestions for this report.

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CORTISOL PRODUCTION AND METABOLISM IN THE NEONATE

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THE adrenal cortex secretes several groups of steroid hormones (Fig. 1) which can be classified into glucocorticoids or corticosteroids, the electrolyte regulating factor which is thought to be aldosterone, and the sex hormones (androgens, estrogens, and progestins). The structure of the steroids isolated from the adrenal vein blood of adult man is shown in Fig. 2. The possible pathways of biosynthesis of progestins, corticosteroids, and androgens are shown in Fig. 3. Aldosterone is thought to be derived from either desoxycorticosterone or corticosterone,[5-7] and estrogens may arise either directly from acetate or from the androgens.[10, 11] Owing to the large scope of the question, we will consider only the secretion of corticosteroids.

Embryologically, the cortex of the adrenal gland is derived from the mesonephros while the medulla is ectodermic in origin.[12] At birth the weight of the adrenals is 7.5 grams, representing about 0.2 per cent of the total body weight, while in the normal adult they correspond to only 0.01 per cent.[13] The weight of the adrenals decreases rapidly after birth due to the reduction in size of the "fetal cortex."[14, 15] This question has been reviewed in detail by Moeri,[16] Lanman,[17] Baar,[18] and Deane.[19] Several studies have suggested that specific zones of the cortex were involved in the production of specific groups of steroids.[20, 21] Owing to the unique structural evolution of the cortex during the newborn period, it is to be expected that the hormonal secretion of the gland during that period would be different from the adult secretion. This subject has been discussed by Lanman,[17] Gardner,[22] and Benirschke and co-workers.[23]

In recent years the development of specific methods for the measurement of steroids in blood and urine has made possible better evaluations of adrenocortical function. However, the determination of the rate of adrenal secretion remains difficult. The ideal technique would be to measure the concentration of steroids in the blood of the efferent vein of the gland, but this procedure is practically impossible in man. Levels of free corticosteroids in plasma can give some indication of the degree of adrenal activity but, as shown in Fig. 1, the plasma levels are not only the reflection of the rate of production but also of

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Fig. 1.—General metabolism of adrenocortical steroids.

Fig. 2.—The different groups of steroids secreted by the adrenal cortex of man. Cortisol, corticosterone, Δ^4-androsterone-3, 17-dione, and 11β-hydroxy- Δ^4-androstene-3, 17-dione have been isolated from the adrenal vein blood of a patient with metastatic carcinoma.[1, 2] Dehydroepiandrosterone has been found in the adrenal vein blood of a patient with virilism,[3] and aldosterone has been identified from dog adrenal blood.[4] Estrogens and progestins are also known to be produced by the adrenal but have not yet been identified.

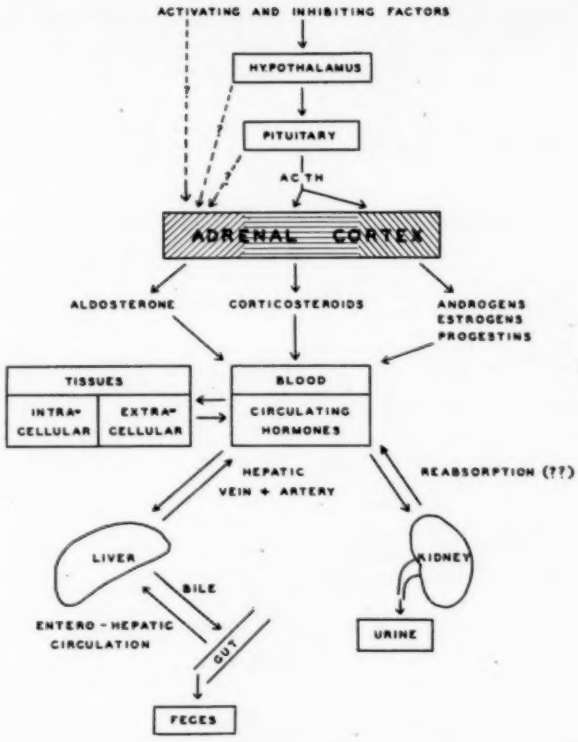


Fig. 1.

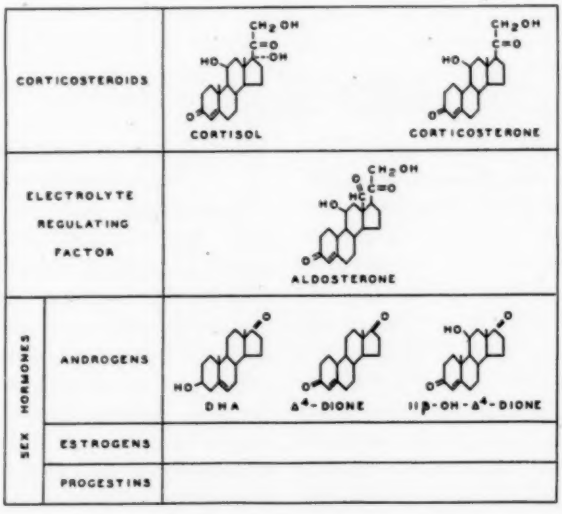


Fig. 2.

Figs. 1 and 2.—*For legends, see facing page.*

the rate of distribution of the hormones in the different tissues and of the rate of destruction of these hormones by the liver. The urinary excretion of corticosteroids will give information on adrenal function if renal function is normal and if the rate of destruction of the hormones by the liver is constant at all times in all conditions. Finally, methods have been devised for the measurement of cortisol productions; they involve the administration of a known amount of radioactive steroid and the determination of isotope dilution in the urine.

It is our purpose in this paper to review the information presently available on the adrenal function during the neonatal period. We will first discuss the problem of corticosteroid production by the neonate. In a second part we will consider the physiologic disposition of these hormones, since in the newborn infant the metabolism of corticosteroids is markedly different from that observed in adult subjects.

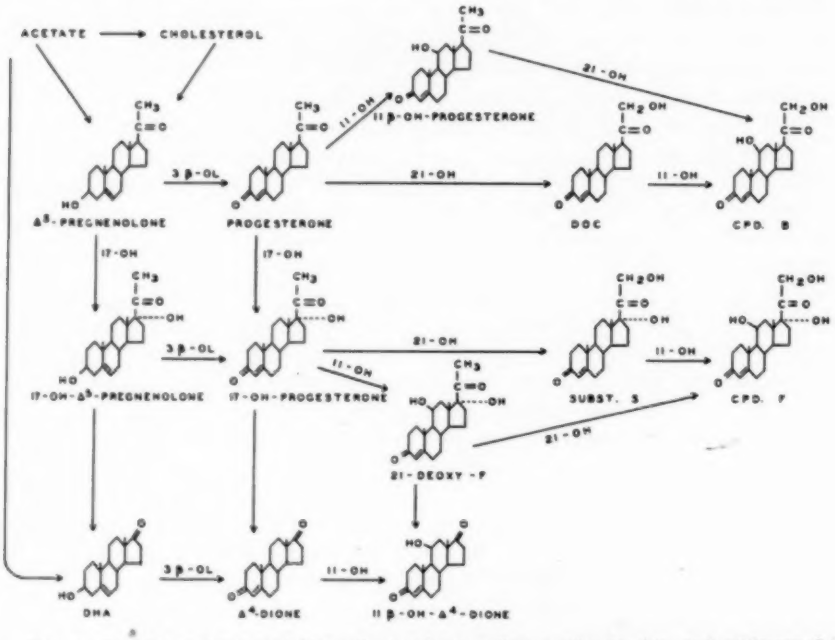


Fig. 3.—Possible pathways of biosynthesis of progestins, corticosteroids, and androgens.[5, 9] Aldosterone is derived from either corticosterone or desoxycorticosterone while estrogens may arise either directly from acetate or from the androgens.

PRODUCTION OF CORTICOSTEROIDS DURING THE NEONATAL PERIOD

Two corticosteroids have been isolated from the adrenal vein blood of man: cortisol and corticosterone.[1] It is generally admitted that cortisol is present in the largest amounts,[1, 24, 25] in addition to being physiologically more active than corticosterone as glucocorticoid.[26] It has been suggested that, during infancy, the concentrations of corticosterone in blood were greater than during adulthood.[27] However, in our experience this steroid was detected

in the plasma of newborn infants and infants only in small amounts and, consequently, we will limit our discussion to cortisol.

Levels of 17-hydroxycorticosteroids in Plasma.—One approach to the study of corticosteroid production is to determine the levels of this steroid in the circulation. The method of Nelson and Samuels[28] as modified by Eik Nes and associates[29] uses the reaction of Porter and Silver[30] and measures steroids with the grouping 17, 21-dihydroxy-20-ketone. This technique will measure not only cortisol but also cortisone, substance S of Reichstein, and their respective dihydro- and tetrahydro- derivatives. However, since cortisone is not physiologically secreted by the adrenal and since substance S is found in human blood only under exceptional conditions,[31, 32] the method of Nelson and Samuels measures mainly cortisol and its reduced derivatives, often referred to as 17-hydroxycorticosteroids (17-OH-CS).

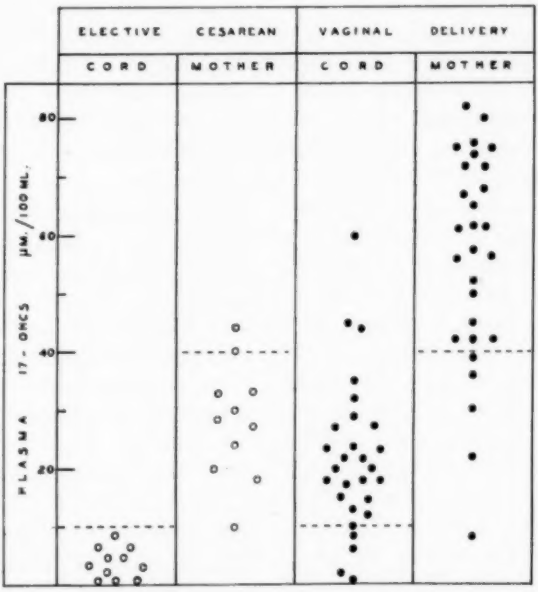


Fig. 4.—Levels of 17-hydroxycorticosteroids in maternal and cord plasma in cases of vaginal delivery and elective cesarean section. The dotted lines mark the 10 μg concentrations for the cord plasma and the 40 μg concentrations for the maternal plasma.

At birth the concentrations of 17-OH-CS in cord plasma are the reflection of the maternal levels. As shown in Fig. 4, in cases of elective cesarean section most of the maternal levels are under 40 μg per 100 ml. of plasma and the levels in cord plasma are under 10 μg; in cases of vaginal delivery most of the maternal levels are over 40 μg while the cord levels exceed 10 μg.[33-35] It has been observed that in most cases the 17-OH-CS concentrations in cord plasma are lower than those of the corresponding maternal values.[34-37] This

finding could be explained if the rate of hormone catabolism by the fetus was more rapid than the rate of passage from the mother to the fetus. This could also be an equilibrium with differences in binding of cortisol to plasma proteins in the maternal and fetal circulation. The latter theory is rather appealing in view of the fact that cortisol has been reported to bind primarily to a specific plasma protein, transcortin, which has been found to be in smaller concentrations in cord blood than in maternal blood.[38, 39]

It has been demonstrated that cortisol can cross the "placental barrier" by the administration of radioactive steroid to pregnant women and subsequent recovery of the steroid in cord blood.[40] In these experiments the ratio of maternal/cord radioactivity was in the same range as that obtained for the 17-OH-CS. This suggests that the corticosteroids detected in cord blood are mainly of maternal origin. However, a minimal production of corticosteroids by the fetal adrenal cannot be excluded and, indeed, in vitro studies have shown that homogenized fetal adrenals incubated with progesterone-4-C^{14} could synthesize corticosteroids.[41, 42] It is not clear, at present, to what extent the fetal adrenal synthesizes corticosteroids. In addition there is some controversy over the question of production of ACTH by the fetal pituitary.[43-45]

It has been reported that after birth the levels of plasma 17-OH-CS fell rapidly to very low levels.[46] It was also found that there was a decreased adrenal response to ACTH stimulation during the first week of life,[47] but when large doses of ACTH were administered, the newborn infant usually had as high a level of circulating free 17-OH-CS as did the older child or adult.[48] At this point a word of caution is probably necessary for the interpretation of the levels of plasma 17-OH-CS during the neonatal period. It has been our experience that interfering chromogenic material is often present in the extracts of samples of plasma of newborn infants. This is also true for the extracts from urine samples; consequently, results reported in this age group must be considered with a certain reserve.

Urinary Excretion of 17-hydroxycorticosteroids.—A second approach to the problem of cortisol production is to measure the amount of cortisol metabolites excreted in the urine over a 24 hour period. In such studies one must be sure that the metabolite or group of metabolites considered are derived only from cortisol. One must also be certain that in the subjects studied cortisol is metabolized in a manner similar to that known to occur in the control subjects. In the present case this is to say that one must be certain that newborn infants metabolize cortisol like normal adults so that a certain amount of urinary metabolites corresponds to a known amount of cortisol production.

Several articles have been published on the excretion of corticosteroids by the newborn infant[49-55] but the methods used lacked specificity and, therefore, the first requirement mentioned above was not fulfilled.

The reaction of Porter and Silber[30] which is known to be quite specific for 17, 21-dihydroxy-20-ketosteroids was used in several studies,[56-59] but, as mentioned above, interfering material is often present in urinary and plasma

extracts. In addition, as will be discussed in the next section on cortisol metabolism, the newborn infant appears to excrete very small amounts of glucuronide conjugates[60, 61] and seems to utilize to a great extent an alternate mode of conjugation of the cortisol metabolites, in contrast to what is observed in normal adults.

It has also been noted that 6β-hydroxy-cortisol was present in relatively large amounts in the free fraction of urinary extracts of young infants.[71]

Production of Cortisol.—An elegant method of measurement of cortisol production has been described by Peterson and Wyngaarden.[62] More recently, Cope and Black[63] have proposed a somewhat simpler technique of estimation of cortisol production in man. Following are the main steps of this technique: A test dose of 4-C^{14}-cortisol of known specific activity (SA_F) is administered to the subject to be studied. All urine is collected for 24 hours following the steroid administration and the total radioactivity contained in this sample (C_U) is determined. The ratio C_U/SA_F gives the weight in micrograms of 4-C^{14}-cortisol present in the urine sample in the form of all radioactive metabolites. The urinary metabolites of cortisol are purified and separated by means of paper chromatography. The specific activity of two of these metabolites, tetrahydrocortisone (SA_{THE}) and tetrahydrocortisol (SA_{THF}), is calculated by dividing the radio-activity present in these fractions by the weight in micrograms. The ratios SA_F/SA_{THE} and SA_F/SA_{THF} should be equal in the limit of the experimental errors, and they represent the degree of dilution of the C^{14}-cortisol metabolites with the metabolites of endogenous cortisol. The total amount of metabolites from cortisol present in the urine sample is given by the formula $C_U/SA_F \times SA_F/SA_{THE}$ which can be simplified into C_U/SA_{THE} (or C_U/SA_{THF} if one considers tetrahydrocortisol instead of tetrahydrocortisone). If from this result one subtracts the total weight of the metabolites of 4-C^{14}-cortisol or C_U/SA_{THF}-C_U/SA_F, one obtains the total amount of metabolites from endogenous cortisol present in the urine sample. However, C_U/SA_F is negligible. Finally, it must be taken into consideration that when a subject is given a test dose of 4-C^{14}-cortisol, 90 to 95 per cent of the dose is excreted in the urine, the balance being excreted in the feces. The technique of Cope and Black is based on a certain number of assumptions: (1) The 4-C^{14}-cortisol administered must be metabolized in the same manner as the endogenous cortisol; (2) cortisol metabolism is similar throughout the 24 hour period of study; (3) tetrahydrocortisone and tetrahydrocortisol must be metabolites of cortisol and only of cortisol; (4) when the specific activity of tetrahydrocortisone and tetrahydrocortisol are determined, these steroids must be separated from impurities which might modify the values of the specific activity. These are reasonable assumptions.

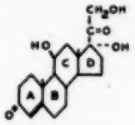
We have used the method of Cope and Black[63] to determine the production of cortisol in three normal newborn infants. Baby AB received C^{14}-cortisol at 15 hours of age, and urines collected between 20 and 42 hours of age were studied. Babies AA and AC received the radiosteroid at 20 hours of age, and the urine study was carried out on samples collected between 29

and 52 hours of age. The urine extracts were prepared as described by Glenn and Nelson.[64] Tetrahydrocortisone and tetrahydrocortisol were separated by paper chromatography using the Bush system,[65] benzene:methanol:water (100:55:45), and eluted from the paper. The radioactivity and the 17-OH-CS content of the eluate were determined and the daily cortisol production was calculated. The results of such calculations were 2.2 mg. per 24 hours for baby AA, 2.6 mg. for AB, and 3.4 mg. for AC. The body surface area of the three newborn infants was approximately 0.2 m^2 and, therefore, the daily production of cortisol per square meter of surface area was 11 to 17 mg. These values compare favorably with those calculated from the data of Cope and Black[63] if one takes 1.5 m^2 for the surface area of the subjects studied by these authors. At the same time, it must be stressed that our newborn infant values might be somewhat erroneous. Since cortisol is metabolized at a slow rate in the neonate, as will be seen below, it is possible that a significant fraction of the tetrahydrocortisone and tetrahydrocortisol measured in the urine in these 3 babies came from the cortisol received from the mother prior to delivery and that our values for production are overestimated. In view of our inability to determine the maternal contribution to the cortisol pool of these newborn infants, the only safe conclusion for our study is that, at least up to 52 hours of age, the neonate is not in a state of hypoadrenocorticism. Further work will be needed to determine cortisol production in older infants when cortisol of maternal origin has been completely excreted.

METABOLISM OF CORTICOSTEROIDS DURING THE NEONATAL PERIOD

Under the stimulation of ACTH from the pituitary gland, the adrenal cortex produces corticosteroids along with sex hormones (Fig. 1). It must be noted that the production of aldosterone is not primarily under the control of ACTH. Once produced, these steroids go into the general circulation and distribute themselves rapidly among the various compartments of the body, especially the extracellular fluid. There is, at present, no proof that cortisol penetrates the cells of any tissues except the cells of the liver.[66] In the liver the steroids undergo important transformations (Fig. 5). The hormones which have been inactivated by the liver and which have been conjugated with either glucuronic or sulfuric acid return to the general circulation, while only a minimal fraction is excreted into the bile.[62, 66, 77] The conjugated steroids of the blood are filtered by the kidney but the free steroids which are loosely bound to plasma proteins[68] and, to a certain extent, to the red blood cells[68, 69] are mostly retained.

The most important transformation that cortisol undergoes in the liver is a reduction in the ring A of the molecule, with formation of an hydroxyl group on the carbon position 3 as shown in Fig. 5. Then an enzyme system, the glucuronosyl transferase, will conjugate the molecule with glucuronic acid.[70] As mentioned above, the steroid can also be conjugated with sulfuric acid. In addition, there appears to be other modes of detoxification as will be discussed later. Other transformations can take place such as



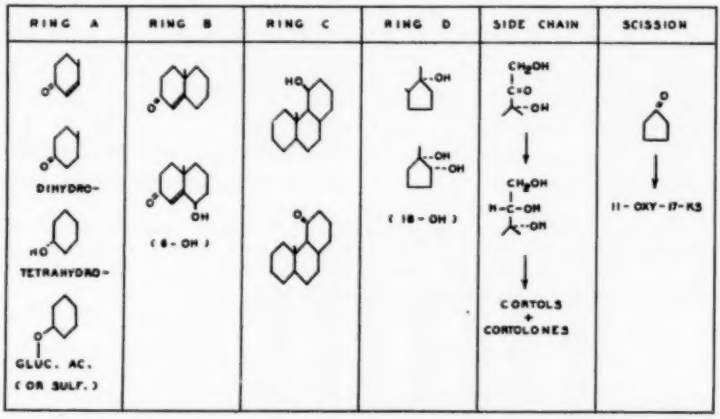


Fig. 5.—Transformations that cortisol can undergo during its metabolism. The reduction of the Δ^4-3-ketone group into a 3-hydroxyl and the conjugation of this 3-hydroxyl group with either glucuronic or sulfuric acid are the most important transformations. The other ones can take place simultaneously. Two to five per cent of a given dose of cortisol is usually excreted in the urine as 17-ketosteroids.

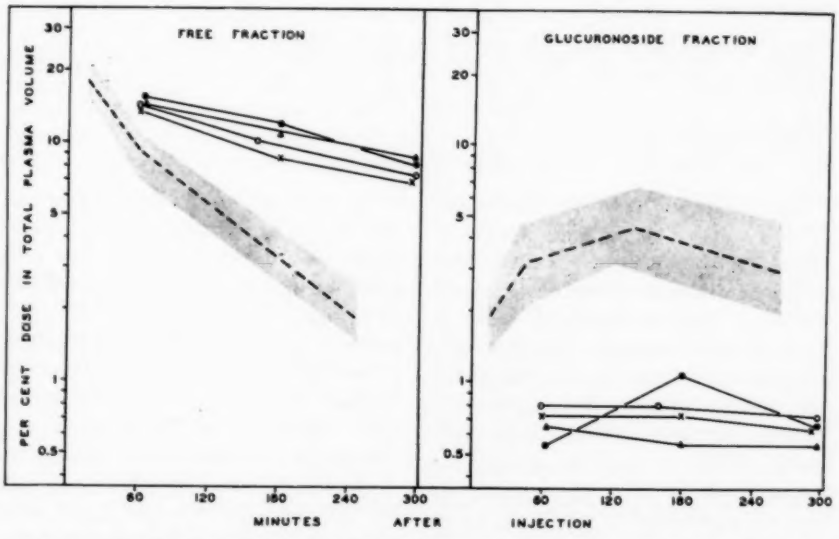


Fig. 6.—Rate of disappearance from the circulation of the free radioactivity following intravenous administration of 4-C^{14}-cortisol to 4 normal newborn infants. These results are compared with those obtained in normal adults.[87]

a reduction of the 20-ketone and the oxidation of the 11-hydroxyl group into a ketone. A 6-hydroxylation and a 16-hydroxylation may also take place. In addition, a small fraction (2 to 5 per cent) of the cortisol is transformed into 11-oxy-17-ketosteroids. The various transformations can take place simultaneously but most of the metabolites will be conjugated in carbon position 3 prior to excretion in the urine.

ance of the free steroid was 2 to 4 times slower than that observed in normal adults. At the same time the radioactivity freed by β-glucuronidase hydrolysis was much lower than that found in normal adult subjects. Some of the values for the half-life of free cortisol reported by Bongiovanni and co-workers were much higher than our values; this might be due to the fact that these investigators administered a comparatively large dose

TABLE I. URINARY EXCRETION OF RADIOACTIVITY BY NEWBORN INFANTS WHOSE MOTHERS RECEIVED AN INTRAVENOUS INJECTION OF 4-C^{14}-CORTISOL FROM 17 MINUTES TO 24 HOURS PRIOR TO DELIVERY BY ELECTIVE CESAREAN SECTION

SUBJECTS	HOURS OF COLLECTION	URINARY EXCRETION (PER CENT DOSE)					
		TOTAL	FREE FORM	GLURONIDE	CONTINUOUS EXTRACTION (pH 0.8)	STRONG-ACID HYDROLYSIS	UNACCOUNTED FOR
Baby A. G. 2,690 grams 904,700 c/m to mother 17 minutes prior to delivery	0 to 24	1.037	0.035	0.102	0.150	0.085	0.665
	24 to 48	0.589	0.009	0.039	0.067	0.034	0.440
Baby A. E. 2,800 grams 907,100 c/m to mother 39 minutes prior to delivery	0 to 24	1.220	0.024	0.048	0.096	0.096	0.956
Baby A. M. 3,200 grams 900,000 c/m to mother 69 minutes prior to delivery	0 to 24	0.022	0.049	0.296	0.390	0.110	1.187
	24 to 48	0.413	0.004	0.034	0.091	0.045	0.239
Baby A. O. 2,810 grams 766,500 c/m to mother 24 hours prior to delivery	0 to 24	0.406	0.012	0.058	0.076	0.022	0.238

Metabolism of Cortisol.—One mode of study of cortisol metabolism is to determine the rate of removal from the circulation of a test dose of the steroid. Bongiovanni and his colleagues[72] have recently reported that in 1-day-old infants the half-life of the free steroid was on the average 977 minutes (range: 250 to 2,100 minutes), while in normal adults the average half-life was 112 minutes. We have obtained similar results in 4 normal newborn infants who had received a test dose of radioactive cortisol. As shown in Fig. 6 the rate of disappear-

of steroid (25 mg. intravenously per infant), while we used 1.3×10^{-2} microcurie of radioactive cortisol per kilogram of body weight, corresponding to a dose of approximately 10 μg of cortisol for a normal newborn infant. In addition, Bongiovanni and his colleagues have carried out their studies with cortisol hemisuccinate; this compound is known to be hydrolyzed very rapidly by adult subjects to give the free steroid, but one might wonder if the hydrolysis might be slower in infants, as suggested by the study of Grumbach and associates.[73]

We have also studied the urinary excretion of the metabolites of cortisol. Table I shows the data obtained in 4 newborn infants. Their mothers had received a test dose of 4-C^{14}-cortisol at various times (17 minutes to 24 hours) prior to delivery by elective cesarean section.[40] As mentioned previously, a certain amount of the cortisol administered to the mother was transferred to the baby. Following delivery, the urine of the newborn infants was collected for 48 hours. If we take the example of Baby A. G. (Table I), it can be seen that during the first 24 hours this infant excreted a total of 1.037 per cent of the dose administered to the mother, of which 0.035 per cent was in the free form, 0.102 per cent was in the glucuronide fraction, 0.105 per cent was in the continuous extraction at pH 0.8 (that is to say the sulfate fraction), and finally, 0.085 per cent of the dose was freed by strong-acid hydrolysis. It will be noted that a large fraction of the total radioactivity was unaccounted for, in this case 0.665 per cent. The same data have been plotted in a different manner as shown in Fig. 7. The results are expressed in per cent of total urinary excretion during the period of collection and are compared with those obtained in normal young adults. In the adults a very small fraction of the total urinary radioactivity was in the free form, while approximately 50 to 55 per cent of the total urinary activity was in the glucuronide fraction. About 8 to 10 per cent was freed by pH 0.8 hydrolysis and strong-acid hydrolysis liberated 4 or 5 per cent, the unaccounted for fraction being approximately 25 to 30 per cent of the total urinary excretion. In contrast, in the various newborn studies there was a marked reduction of the glucuronide fraction, while the free fraction, the sulfate fraction, and the fraction freed by strong-acid hydrolysis were not significantly different from those found in normal adults. Consequently, in all cases there was a large increase in the unaccounted-for fraction.

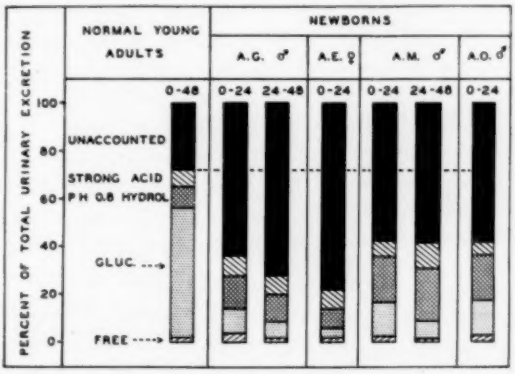


Fig. 7.—Fractionation of the radioactivity excreted in the urine of the newborn infants whose mothers received an intravenous injection of 4-C^{14}-cortisol from 17 minutes to 24 hours prior to delivery by elective cesarean section. The fractions are expressed in per cent of the total radioactivity excreted in the corresponding urine specimen.

Similar results were obtained when 4-C^{14}-cortisol was administered directly to the newborn infant instead of being given to the mother prior to delivery. The cumulative urinary excretion of radioactivity by baby AB (15 hours of age) and baby AC (20 hours of age) is shown in Fig. 8. After a period of slow rate of excretion during the first 8 hours, the rate was similar to that observed in normal adults so that at the end of 48 hours the total excretion was not significantly different from that observed in adults. The glucuronide fraction of these urines was small while the unaccounted-for fraction was very large (Fig. 9), as was found in the urinary

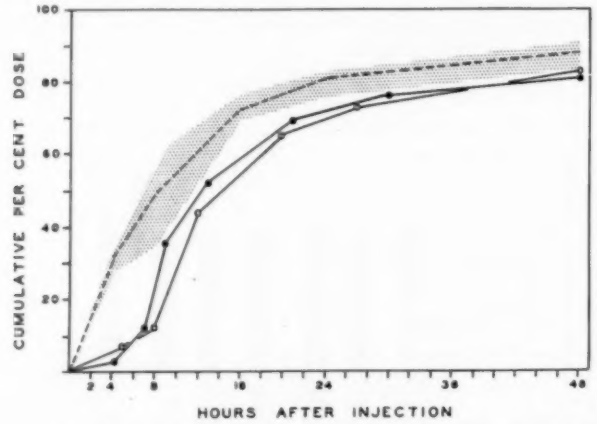


Fig. 8.—Cumulative excretion of radioactivity by two normal newborn infants who received an intravenous injection of 4-C^{14}-cortisol. The dotted line represents the average excretion by normal adults, the shaded area shows the range of variation. The full lines show the excretion by the two newborn infants.

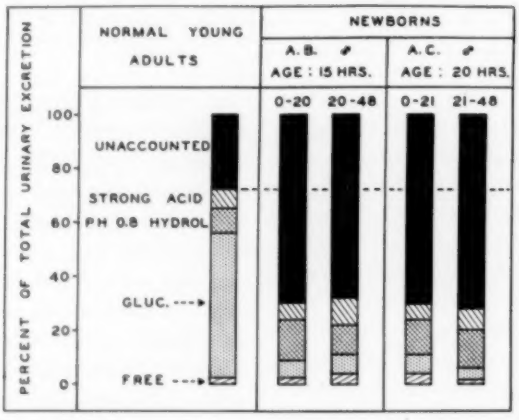


Fig. 9.—Fractionation of the radioactivity excreted in the urine of two newborn infants who received a test dose of 4-C^{14}-cortisol. The fractions are expressed in per cent of the total activity excreted in the corresponding urine specimen.

studies in newborn infants whose mothers had received the radioactive cortisol.

Metabolism of Tetrahydrocortisone.—Bongiovanni and collaborators[72] have reported that tetrahydrocortisone's rate of disappearance from the circulation was very rapid in two 1-day-old infants, being comparable to that observed in two older children and in adult individuals.[74] These data suggest that in the newborn infant the enzymes involved in the conjugation of tetrahydrocortisone with glucuronic acid are functionally normal. However, in our experience[60] such a conclusion does not appear to be justified. Several newborn infants were given an intravenous infusion of tetrahydrocortisone (2 mg. per kilogram of body weight), and the 17-OH-CS excreted in the urine during the 24 hours following this procedure were measured. In normal adults approximately 60 per cent of the administered tetrahydrocortisone is recovered as glucuronides, while in the newborn infants only 10 to 20 per cent was recovered in this fraction.

DISCUSSION AND CONCLUSIONS

It has been demonstrated beyond any doubt that cortisol can cross the "placental barrier" and there are reasons to believe that cortisol can be returned by the fetus to the mother. The apparent gradient between maternal and cord levels is probably only the resultant of a complex two-way transfer.

Pregnancy is known to increase the concentrations of 17-OH-CS in the maternal plasma. The processes of labor and delivery by the vaginal route raise further the levels, while the process of elective cesarean section has much less immediate effect on the levels. Since the concentrations of 17-OH-CS at birth are much greater in cases of vaginal delivery than in cases of elective cesarean section, it has been suggested that the cortisol which is detected in cord blood is mainly of maternal origin. There is evidence to suggest that fetal adrenal has the potentiality of secreting cortisol but it seems that the fetus produces only moderate amounts of this steroid.

We have shown that the 17-OH-CS excretion in urine of newborn infants would give a very erroneous picture of the production of cortisol because of the marked alteration of the metabolism of the steroid. Our study of cortisol production in 3 newborn infants revealed values at least equal to or greater than those reported for adults when the results were corrected for body surface area. However, part of the cortisol pool of these newborn infants was probably of maternal origin and the values for production were overestimated. In any case, it can be said that the neonate does not lack corticosteroids, at least during the first 2 days of life.

Tetrahydrocortisone and tetrahydrocortisol are, quantitatively, important metabolites of cortisol, and their glucuronide conjugates are found in large amounts in the urine following cortisol administration.[75] In the neonatal period the enzyme systems involved in the catabolism of cortisol appear to be less active than in adult subjects, as demonstrated by the prolonged half-life of this steroid. The finding of a normal half-life for tetrahydrocortisone has suggested that the

slow step in the degradation of cortisol was at the level of the enzymes which are involved in the reduction of the Δ^4-3 ketone rather than at the level of the enzymes which conjugate a 3-hydroxyl group with glucuronic acid.[63] However, the administration of tetrahydrocortisone to newborn infants was found to yield very little glucuronide conjugates in urine when compared to results obtained in adult subjects and, therefore, the glucuronyl transferase is also markedly inactive in the neonatal period. This latter enzyme is also involved in the conjugation of bilirubin with glucuronic acid which has been shown to be decreased in the newborn infant.[76]

Finally, it is of interest that the neonate uses, to a large extent, one or several alternate modes of catabolism of cortisol. The study of a child with familial nonhemolytic jaundice[77] has shown that this patient excreted a large proportion of a test dose of 4-C^{14}-cortisol in the unaccounted-for fraction (Fig. 10). Whether the unaccounted fraction of adults, of newborn infants, and of this patient with familial nonhemolytic jaundice is made up of the same cortisol metabolites remains to be determined.

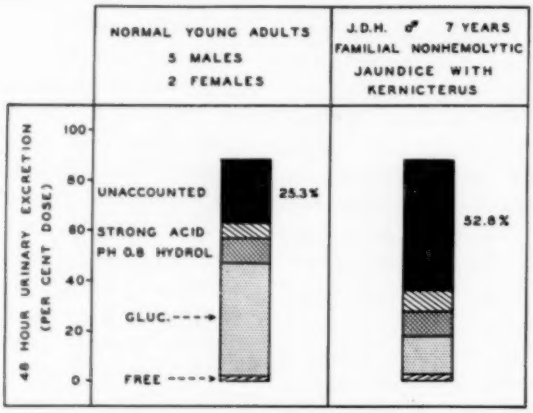


Fig. 10.—Fractionation of the radioactivity excreted in the urine of a child with familial nonhemolytic jaundice who received a test dose of 4-C^{14}-cortisol.

It might be concluded, from the data presently available, that the rate of cortisol production of the newborn infant in either physiologic or pathologic conditions can only be speculated upon and not definitively determined.

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POTASSIUM DEFICIENCY AND ADRENOCORTICAL HORMONES IN THE ETIOLOGY OF METABOLIC ALKALOSIS

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DARROW and co-workers[1] in 1948 reported a series of experiments defining an interesting electrolyte syndrome which correlated changes between intracellular cations and extracellular anions. With reference particularly to states of potassium deficiency, they noted an association between a loss of potassium and gain of sodium by the cells, with a decrease in the chloride and increase in the bicarbonate in the extracellular fluids. This syndrome is diagrammed in Fig. 1. Darrow's findings created immediate excitement; in subsequent years many publications on this topic have appeared, all of which have been confirmatory to his original observations.

IMPORTANCE OF THREE ORGAN SYSTEMS IN THE GENESIS OF THE DARROW SYNDROME

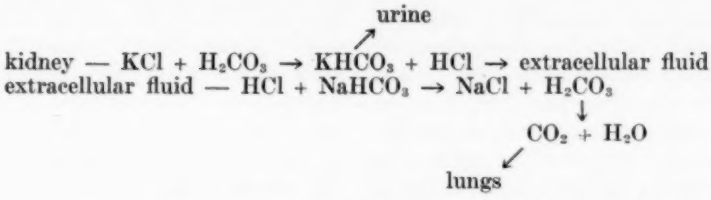
Darrow did not speculate in his original article as to the nature of the underlying mechanism other than to point out that the electrolytes of the intra- and extracellular fluids appear to be related by some sort of biologic equilibrium. In the intervening years, however, continuing investigation of this problem has revealed the importance of three different organ systems. It has been emphasized that certain known patterns of renal response are those that would contribute to the enhancement of the "Darrow shift."[2] Other findings point to the importance of ion exchanges across the muscle cell membrane[4]; finally, recent observations[5] indicate adrenocortical activity to be an important factor in the genesis of this electrolyte syndrome.

The present discussion will attempt to analyze the contributions of these separate systems in a way that will aid in the understanding of the Darrow syndrome as it is encountered clinically. It is simplest to divide the problem in terms of acute and chronic conditions.

ACUTE CONDITIONS

It is when dealing with acute or changing conditions that the ion-exchange theories have proved most useful to explain linkage between potassium metabolism and acid-base balance.

The studies of Loeb and associates[6] of the response of the kidney to potassium chloride administration are considered first. These authors gave 200 mEq. of KCl to a human subject. Subsequent balance measurements indicated that 71 per cent of the potassium and 51 per cent of the chloride were eliminated during the first day. Twenty per cent of the administered KCl can be considered to have reacted in the following way:



Excess potassium appeared in an alkaline urine as $KHCO_3$ and extra chloride remained in the extracellular fluid in association with a decrease of the bicarbonate and extracellular pH. Kennedy, and Orloff[3] particularly noted the inverse correlation between the excretion of potassium and the excretion of hydrogen. They postulated that these ions compete with

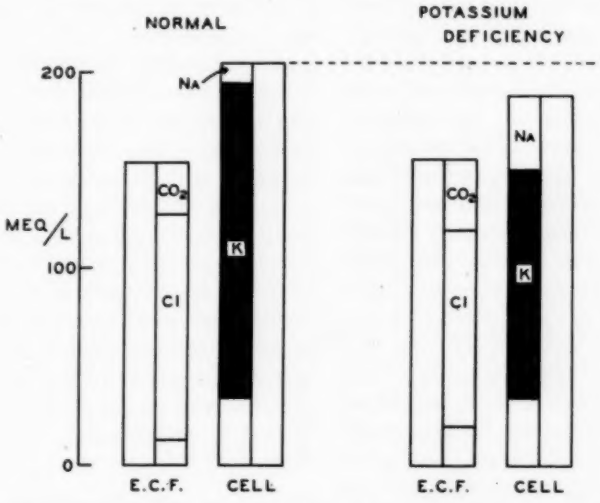


Fig. 1.—Diagrammatic representation of the "Darrow shift," showing the changes of the intracellular cations and extracellular anions. (Data modified from Cotlove, Holliday, Schwartz, and Wallace.[2])

In terms of hydrogen and potassium an increased excretion of potassium was associated with a decreased hydrogen loss and an increased delivery of hydrogen to the extracellular fluid.

Stanbury and Thomson[7] and Franglen and co-workers[8] have observed in examples of acutely induced alkalosis a spontaneous increase in the excretion of potassium. In an analysis of these and other findings Berliner, each other for access to a common renal tubular secretory mechanism which presumably involves exchange with sodium from the glomerular filtrate. Physiologists have found this postulation a useful one to aid in the prediction of renal response to acute variations in either electrolyte load or of serum acidity.

Cooke, Segar, Cheek, Coville, and Darrow[4] in studies of the low potassium

syndrome concentrated their attention particularly upon the biochemical lesion of the muscle cell. They point out that potassium lost from the muscle in deficiency states is incompletely replaced by sodium (see Fig. 1). On a quantitative basis only 2 mEq. of sodium enter the cell for every 3 mEq. of potassium lost. Since the law of electroneutrality must be fulfilled, another positively charged ion must enter the cells. This cation is presumed to be hydrogen. These authors[4] were able to visualize in a stepwise fashion the dynamic aspects of the change. Since induction of the syndrome requires weeks, the observations were carried out instead on the recovery process as observed with potassium replenishment. The data were indicative of an emergence of hydrogen from the cell which could account for the measured decrease in the extracellular bicarbonate.

$$\frac{H^+}{\text{cell}} \rightarrow + HCO^-_3 \rightarrow H_2CO_3 \rightarrow H_2O + CO_2$$

This interpretation has been subscribed to by Orloff, Kennedy, and Berliner[9] who demonstrated the same mechanics of recovery in the nephrectomized rat.

From a clinical standpoint these findings are significant since they show that potassium deficiency alkalosis can be corrected with a neutral salt, KCl, without supply of additional chloride or hydrogen (NH_4). The extracellular bicarbonate will return to normal in association with the release of cellular hydrogen without requiring supplementary corrective action on the part of the kidney. The sequence of events may also be expressed in terms of sodium, potassium, and chloride. Following the administration of 3 U. of potassium and 3 of chloride, the 3 U. of potassium will enter the cell but only 2 of sodium will emerge. The net result will be the addition of 3 U. of chloride and 2 of sodium to the extracellular fluid. The relative gain in the fixed anion column is equivalent to the emergence of hydrogen from the cell and equivalent to the reduction of the extracellular bicarbonate.

Exchange of hydrogen for potassium across the cell wall can be related in a quantitative fashion to the acidity of the extracellular fluids. Intracellularly, on the other hand, the nature of the buffers is incompletely understood and the change cannot be predicted accurately. Experimental measurements of the pH of muscle tissue in potassium-depleted animals showed a lowering of 0.6 pH unit in one study[10] and 0.06 pH unit in another.[11] Since a newly formed intracellular hydrogen (buffer anion) system cannot be considered as being immune to metabolism and breakdown to CO_2 and H_2O, the presence of the added hydrogen cannot be stipulated theoretically on more than a temporary basis.

CHRONIC CONDITIONS

Concepts involving ion exchanges are useful to describe the acutely changing state, but these postulations do not explain maintenance of the steady state. Exchanges of ions between the intra- and extracellular compartments affect the composition of both, but the actual quantities involved are not large enough to bring about more than transient alterations in the extracellular acidity in normal subjects.

The studies of Loeb and co-workers[6] of the effects of KCl loads were not

confined to observations of the acute phenomenon. In these experiments a high intake of KCl was maintained over a 48 hour period. The changes of the first day were those described in the preceding section. On the second day the relative quantities of potassium and chloride excreted were found to be just the reverse of those measured for the first day. At the end of the 2 days the balance of the 2 ions was essentially the same and no acid-base derangement was present. Hence, it is important to describe the acidifying action of suddenly presented loads of KCl as a tendency, one that will be overcome under normal circumstances.

Since the volume of the extracellular fluid filtered and reabsorbed is so large, the composition is inevitably resultant upon the constitution of the reabsorbate. Chronic alkalosis must be due to some metabolic disorder in certain renal tubular cells that are responsible for regulation of acid-base balance. The roles of adrenocortical hyperactivity and of potassium deficiency in the genesis of this disorder are reviewed next.

Grollman and Gamble[5] showed that alkalosis induced by the adrenal cortical hormones in dogs would subside if the hormones were withdrawn even during restriction of dietary potassium. These experiments indicate a direct action of the hormones, for they demonstrated induction of and recovery from alkalosis in the absence of significant alterations in potassium metabolism. Under the more usual conditions, however, administration of the adrenal hormones is associated simultaneously with developing alkalosis and an increased excretion of potassium. It is obvious that this dual capacity enables the adrenals to play an important role in the induction and maintenance of the Darrow syndrome. This dual action of the hormones can be implicated in the etiology of the alkalosis of Cushing's disease and of that associated with administration of large therapeutic doses of exogenous hormone. There is now well-established evidence[12] of a large release of 17-hydroxycorticoids following major operations, and there is every reason to believe that this discharge is important in the genesis of the metabolic alkalosis observed in certain severely ill postoperative patients.

Recognition of the specific alkalinizing tendency of the adrenocortical hormones invites some re-evaluation of the role of potassium deficiency per se as the cause for chronically maintained metabolic alkalosis. Darrow and associates[1] have shown that dietary deficiency of potassium will in itself bring on alkalosis in the intact rat, but these studies do not rule out the possibility of an endogenous increase in hormone excretion in the severely depleted animals. One would not expect a low potassium diet to be a physiologic stimulus for release of mineralocorticoid (aldosterone), but an alarm-type reaction in a severely ill animal would be expected to increase the secretion of glucocorticoids. Histologic and physiologic evidence of adrenal hyperactivity in potassium deficiency has been reported.[13, 14] At any rate, a clear-cut relationship between potassium depletion and alkalosis has not been described in adrenalectomized animals receiving no more than maintenance doses of cortical hormones.

Despite the questions just raised as to the role of potassium deficiency in the etiology of alkalosis, there is no doubt that a high potassium intake will effectively prevent the onset of alkalosis in the presence of increased adrenocortical activity of either exogenous or of endogenous origin. Three milliequivalents per kilogram each day has been recommended for this purpose.[15] It is not clear as to whether the effectiveness of this relatively high dose is to be interpreted in terms of a measure to prevent formation of a cellular deficit or whether it is related in some way to increased transcellular movement of potassium through certain cells of the renal tubules. It does appear that the actual flux of potassium through these cells, that is, the amount secreted, is important in the conditioning of the cells to raise resistance to the action of the adrenocortical hormones. It is interesting that the high potassium intake will control the alkalinizing tendency of the hormones on a chronic basis. This is in contrast with the transient nature of the acidifying effect of high KCl intakes in normal subjects. One might consider the acidifying tendency of KCl loads as dominant to the alkalinizing tendency of the hormones but subservient to the homeostatic mechanisms in normal man.

At the present time the state of our knowledge does not permit definition of the action of the hormones or of potassium deficiency at the biochemical level. It is obvious that any cell depleted of its native cation is an abnormal cell and that deviations from the normal activity patterns would be expected. Cooke and co-workers[16] carried out a series of experiments designed to elucidate the defect of potassium deficiency as it becomes manifested by errors of renal regulations. Particularly they noted critical limitations in the ability to retain chloride. These findings were supplemented with the observation that a normal response to administration of alkali, namely, increased excretion of citrate, did not appear until the potassium deficiency was repaired. It was suggested that reduction in the efficiency of chloride retention is due in part to an inability to produce an organic anion to serve as a substitute for excretion. The data relate an abnormality of Kreb's cycle activity to the potassium deficient state. Effects of adrenocortical hyperactivity upon these relationships have not been fully investigated. Recent studies by Grollman and Harrison* suggest limitations in citrate reabsorption in the presence of an alkaline urine but data are not available to explain adequately abnormal regulation and excessive chloride loss in states of potassium deficiency.

TABLE I. RELATIONSHIPS BETWEEN ADRENOCORTICAL STATUS, SODIUM AND POTASSIUM METABOLISM, AND EXTRACELLULAR pH

	ADRENAL STATUS		
	HYPO	NORMAL	HYPER
pH	Low	Normal	High
Total body K	Increased	Normal	Decreased
Total body Na	Decreased	Normal	Increased
High K intake	Toxic	Tolerated	Therapeutic
High Na intake	Therapeutic	Tolerated	Toxic

*Grollman, A. P., and Harrison, H. E.: Personal communication.

SPECTRUM OF INTERRELATIONSHIPS BETWEEN ADRENAL ACTIVITY, SODIUM AND POTASSIUM INTAKE, AND pH

Sodium metabolism is an additional parameter of well-recognized importance in the genesis of the changes of the Darrow syndrome.[17, 18] One more factor would seem to render the overall picture hopelessly complex. Fortunately, however, the interrelationships between sodium and potassium metabolism and adrenal activity can be represented in an orderly fashion as shown in Table I. Throughout the range of adrenocortical activity the effect of a high sodium intake is synergistic to the action of the hormone with reference to pH while the effect of a high potassium intake is consistently antagonistic.

POTASSIUM THERAPY

In summary and with reference to therapy, it is clear that potassium must be recognized as a many-toothed weapon in the treatment of the Darrow syndrome.

Potassium administered acutely as a neutral salt will have a transient acidifying affect upon the extracellular fluids. In normal man this effect is mediated by the kidney. In the treatment of the potassium deficient subject the effect is mediated in addition by ion exchanges occurring between cells and extracellular fluids.

Potassium therapy will quickly repair an intracellular deficiency of potassium and this event can be expected to correct any associated biochemical defects and consequent abnormalities with relation to physiologic function.

Under chronic conditions a high intake of potassium will protect against the potassium depletion action and alkalinizing effects of excess adrenocortical activity.

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THE CLINICAL SIGNIFICANCE OF AMINOACIDURIA

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THE excessive excretion of amino acids in the urine is significant as a manifestation of aberrant cellular function. It may result from deranged intermediary amino acid metabolism in various organs and from impaired renal tubular reabsorption. In clinical problems patterns of urinary amino acid excretion may provide essential clues in diagnosis and in genetic surveys and in the elucidation of metabolic defects occurring in obscure syndromes. Currently, aminoaciduria is being intensively studied in renal tubular diseases and in clinical syndromes in which abnormal brain function predominates. Excellent reviews of the clinical and other aspects of syndromes associated with abnormal amino acid excretion are available.[1-5] The significance of the phenomenon itself will be discussed here in terms of the renal tubular handling of amino acids.

At the outset it should be stated that hyperaminoaciduria does not lead to amino acid depletion in the nutritional sense. Childs[6] has found that normal children excrete approximately 2.5 mg. total α-amino acids per kilogram per day, a minute quantity in comparison with the usual protein intake. In those disorders in which aminoaciduria is often described as "massive" or "severe," the loss of amino acids in the urine may exceed normal quantities by 10 to 50 fold. Even so, such a loss would be nutritionally significant only in the presence of grossly deficient intake. In cystinuria the greatly elevated concentrations of cystine in the urine may promote calculus formation. With this exception, the presence of the various amino acids in excessive amounts in the urine is not known to be deleterious. Hyperaminoaciduria, per se, requires no therapy; however, it is frequently associated with other derangements of cellular function, many of which are responsive to appropriate therapy. Some of these disorders have similar clinical manifestations, but they may be differentiated by the distinctive urinary amino acid excretion pattern with which each is associated. Herein lies the clinical usefulness of urinary amino acid studies.

In order to evaluate amino acid studies one must be aware of the limitations inherent in the various analytic methods used. Precise and accurate methods are available for the quantitative determination of total α-amino acids.[7] These methods do not provide information about the concentrations of individual amino acids in a sample. Differences in the relative concentrations of various amino acids one to another are clinically and functionally important. These differences

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give rise to distinctive "patterns of aminoaciduria." Paper chromatography, a technique devised by Consden, Gordon, and Martin[8] and adapted by Dent[9] for analysis of complex biologic fluids, possesses the capacity to delineate qualitative differences between these patterns. This technique has the advantage of simplicity, rapidity, and ease of application to a large number of samples. It is particularly suited to the analysis of urine in which the deviations from normal are often great. The semiquantitative nature of the technique greatly limits its usefulness in the analysis of plasma. For precise quantitative determination of individual amino acid concentrations in plasma and urine, one must employ ion-exchange chromatographic,[10] microbiologic,[11] and enzymatic techniques.[12] These methods present greater technical difficulties. In clinical research they find their chief value in the substantiation and amplification of information obtained by paper chromatography.

Interpretation of patterns of hyperaminoaciduria must take cognizance of normal reabsorption of each amino acid by intact renal tubules. The studies of Doolan and associates[11] and Evered[13] in normal subjects clearly demonstrate that not only are the concentrations of individual amino acids in plasma different but so also in an unrelated manner are their individual endogenous clearances. The relative concentrations of each amino acid in the urine reflect these differences. This is shown in Fig. 1 which is adapted from the data of Doolan and colleagues.[11] For example, the tubular reabsorption of glycine and histidine is not quite as complete as that of other amino acids. These two amino acids are normally found in the greatest concentrations in urine. Since the plasma glycine concentration is the greater of the two, its concentration in urine is normally greater. On the other hand, the plasma concentrations of glycine and alanine are equivalent, but the concentration of alanine in the urine is much less than that of glycine. This is so because the clearance of alanine is normally about one fourth to one eighth that of glycine. The tubular reabsorption of other amino acids such as leucine, methionine, and arginine is virtually complete, so that these appear in the urine in infinitesimal quantities. The data shown in Fig. 1 are based upon studies in adults. The findings are, however, representative of data obtained in children.[14] Only in premature and young full-term infants is the normal pattern of amino acid excretion both qualitatively and quantitatively different.[6, 15, 16] These differences disappear during the latter one half of the first year of life and can usually be distinguished from the abnormal patterns of aminoaciduria which will be discussed.

The data shown in Fig. 1 depict the typical pattern of amino acid excretion in the fasting state. Ordinarily, diet has little influence on amino acid excretion. In patients with severe malnutrition Westall and co-workers[17] have found that plasma amino acid concentrations are significantly lowered. Similar precise studies of urinary excretion in malnutrition have not been reported,* although an increased excretion of β-aminoisobutyric

*Since the preparation of this manuscript, Schendel and co-workers (Pediatrics 23: 662, 1959) have reported the occurrence of increased amino acid excretion in patients with kwashiorkor. The pattern described is that of a generalized renal aminoaciduria. It cannot be determined from the data whether severe malnutrition, per se, or the electrolyte disturbance found in this syndrome is primarily responsible for the aminoaciduria.

acid during acute starvation has been observed.[18] When dietary protein intake is increased above the usual amounts, no over-all increase in urinary amino acid excretion occurs.[19, 20] Exceptions to this are the teral administration of protein hydrolysates in the usual therapeutic manner does not elevate plasma amino acid concentrations enough to cause a significant increase in their excretion in the urine. On the one hand, efficient

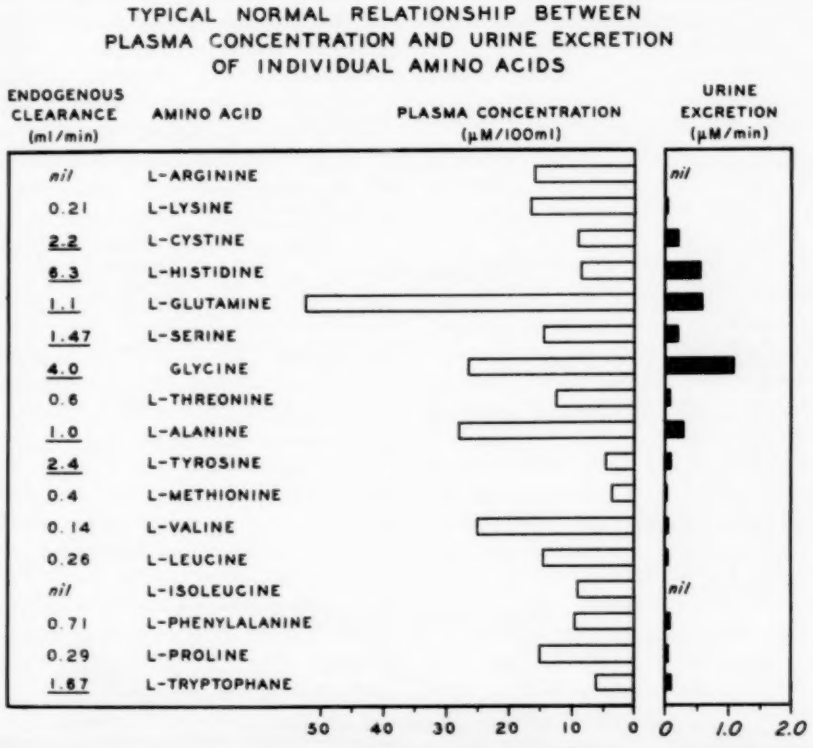

Fig. 1.—The plasma concentrations and urine excretion of the amino acids shown are redrawn from the data of Doolan and colleagues.[11] These are fasting values obtained during the course of endogenous renal clearance studies in a healthy young adult man. Since the measured glomerular filtration rate in this case is 112 ml. per minute, the plasma concentrations (μM. per 100 ml.) approximate the filtered load (μM. per min.). Note that the scale for urine excretion is 10 times the scale for plasma concentration. Note further that the tubular reabsorption of all amino acids is virtually complete so that only a small fraction of the filtered load of each is actually excreted in the urine. Glycine, the amino acid whose excretion is the greatest, is actually 96 per cent reabsorbed. There is considerable variation in both plasma concentrations and endogenous clearance rates. These independent variables account for the fact that the normal distribution of amino acids in the urine is quite different from that in the plasma.

increased excretion of histidine,[19] taurine, and methylhistidine[21] with liberal protein intake. The increased excretion of these three amino acids alone should be viewed as variations in the normal pattern. Likewise, the parendeamination of amino acids by the liver prevents significant elevation of their concentrations in plasma; while, on the other, virtually complete renal tubular reabsorption of amino acids prevents diminution of their plasma

concentrations. Thus, both renal and hepatic factors are important in the normal stabilization of plasma amino acid concentrations.

If, however, plasma amino acid concentrations are significantly elevated by continuous intravenous infusion of hypertonic amino acid solutions, both the amounts reabsorbed by the tubules and the amounts excreted will be increased.[11] If the infusion mixture simulates the normal distribution of amino acids in plasma, a generalized "overflow" aminoaciduria will result. Similarly, if the plasma concentration of a single amino acid is sufficiently raised, its excretion in the urine will be increased but the plasma levels and urine excretion of other amino acids may be little affected. Hyperaminoacidemic or "overflow" aminoaciduria is analogous to the glycosuria which occurs in the presence of hyperglycemia. Conversely, renal aminoaciduria occurs in the presence of normal or reduced plasma concentrations as a result of reduced renal tubular reabsorptive capacity for amino acids. This is analogous to the causation of renal glycosuria. These physiologic considerations prompted Dent and Walshe[22] to classify pathologic hyperaminoacidurias in the four groups which are shown in Table I.

TABLE I. TYPES OF AMINOACIDURIA

"OVERFLOW" AMINOACIDURIA	
GENERALIZED	SPECIFIC
Severe liver disease[33]	Phenylketonuria[34] Hyperglycinuria[35] *(Hypophosphatasia)[36] *(Argininosuccinicaciduria)[37, 38] "Maple syrup urine disease"[53, 54]
RENAL AMINOACIDURIA	
GENERALIZED	SPECIFIC
Cystinosis[23] Fanconi syndrome (infantile[1] and adult[24] forms) Vitamin D deficiency states[45, 46] Heavy metal intoxications (lead, cadmium, uranium, mercury)[43, 48, 49] Other poisonings (lysol)[25] Wilson's disease[50, 51] Galactosemia[52] Congenital renal tubular acidosis[44] Luder-Sheldon syndrome[26] Multiple myeloma[27] Glycogen storage disease[28] Lowe syndrome[29]	Cystinuria[39] β-aminoisobutyricaciduria[40] Hartnup syndrome[41] Vitamin C deficiency[14] (generalized plus tyrosine and ?phenylalanine) Simple glycinuria[42]
AMINOACIDURIAS (UNCLASSIFIED)	
GENERALIZED	SPECIFIC
Nephrosis[30] Increased protein catabolism[31, 32] (i.e., burns, cortisone, muscle degeneration, etc.)	Vitamin D resistant osteomalacia with hyperglycinuria[4]

This classification of aminoacidurias follows that originally proposed by Dent and Walshe.[22] The basis of this classification is discussed in the text. The syndromes listed are thoroughly discussed in the references cited. Wallis and Engle[24] list additional reports of aminoaciduria.
*Substance excreted is not an α-amino acid but a closely related compound (see text).

These groups are: generalized "overflow" aminoaciduria, specific "overflow" aminoaciduria, generalized renal aminoaciduria, and specific renal aminoaciduria.

Since the liver normally prevents an appreciable rise in plasma amino acid concentrations, it follows that generalized "overflow" hyperaminoaciduria usually indicates severe hepatic cell injury without regard to the etiology of the liver disease. The pattern of generalized hyperaminoaciduria characteristic of hepatic disease can be distinguished from that of generalized renal hyperaminoaciduria by the fact that the urine concentrations of the sulfur-containing amino acids (taurine, cystine, and methionine), ethanolamine, and β-aminoisobutyric acid are greater in hepatic disease.[22, 33]

Specific "overflow" aminoacidurias result when the plasma concentrations of one or a few amino acids are elevated. In phenylketonuria both plasma and urine concentrations of phenylalanine are raised, but those of the other amino acids remain unaltered.[13, 34] We have recently observed a patient with clinical abnormalities referable to many systems. He excretes excessive quantities of glycine when his plasma glycine concentration is raised. Other urinary amino acid concentrations are apparently normal in this patient. The nature of his metabolic disorder is currently being studied.[35]

In other disorders metabolic intermediates not normally detected in the extracellular fluid may appear in low concentration in the plasma. These apparently are not appreciably reabsorbed from the renal tubular fluid and therefore are found in relatively high concentration in the urine. Intermediates which are closely related to α-amino acids chemically may react to give a similar colored product with ninhydrin, the most widely used reagent for amino acid identification. Examples of this are the excessive excretion of phosphoethanolamine in hypophosphatasia[36] and of argininosuccinic acid[37] in a recently reported sibship manifesting mental deficiency.[38]

The specific "overflow" aminoacidurias are to be distinguished from specific aminoacidurias in which defective renal tubular reabsorption is the principal factor. Best understood in this group is cystinuria,[39] in which the plasma concentrations of lysine, arginine, cystine, and ornithine are normal but their reabsorption is impaired. Other amino acids are present in both plasma and urine in normal amounts. The renal defect, therefore, is apparently limited to a step in the reabsorptive pathway shared by and essential for the reabsorption of lysine, arginine, cystine, and ornithine but not essential for the reabsorption of other amino acids. Genetic studies have revealed that certain individuals excrete an excessive quantity of β-aminoisobutyric acid[40] in the presence of normal plasma concentrations of this and other amino acids.[13] The defect is apparently renal but of no clinical consequence since β-aminoisobutyric acid is an end product of pyrimidine metabolism. Patients with the "Hartnup syndrome"[41] exhibit a complex hyperaminoaciduria in which the endogenous renal clearances of serine, threonine, asparagine, histidine, and tyrosine are increased to an extent far out of proportion to increases in the clearances of other amino acids.[13] Although the aminoaciduria is apparently

on a renal basis, the manifold clinical features, which simulate pellagra, suggest that the metabolic defect is present in other organs as well as in the kidney.

It is usually possible to distinguish between generalized aminoacidurias of the "overflow" and renal types on the basis of urinary patterns alone. Plasma as well as urine studies are required to differentiate specific aminoacidurias. Hyperglycinuria illustrates this point. Three distinct clinical entities associated with hyperglycinuria are presently recognized. In the syndrome described by Dent and Harris[4] as "vitamin D resistant osteomalacia with hyperglycinuria," the urine concentrations of glycine and (to a lesser degree) of proline and l-methylhistidine are elevated.[13] Until precise clearance studies are available, it cannot be decided whether this specific aminoaciduria results from "overflow" or impaired renal tubular reabsorption. Mentioned earlier was an "overflow" hyperglycinuria. This patient exhibits abnormalities referable to many systems, suggesting a widespread defect in glycine metabolism.[35] DeVries and co-workers[42] have described an hereditary disorder associated with nephrolithiasis in which the hyperglycinuria is renal in origin. Determination of whether a given hyperaminoaciduria is renal or extrarenal in origin is important in ascertaining the nature of the underlying metabolic defect.

In this laboratory considerable attention has been devoted to the study of generalized renal aminoaciduria[43] in which the immediate cause is impaired tubular reabsorption. This is not to say that disorders characterized by generalized renal hyperaminoaciduria (Table I) are limited to the renal tubules. On the contrary, many of these disorders have manifestations referable to other organs, particularly the liver and the brain. Reports in the literature, which often have been based on single specimens from small groups of patients, might suggest to the reader that there are specific patterns of aminoaciduria for some of these diseases. Semiquantitative paper chromatographic studies in this laboratory suggest that these differences are quantitative rather than qualitative.[43] This qualitative similarity becomes apparent when the patterns found in various disorders are arranged in order of increasing over-all severity and irrespective of clinical diagnosis. This is shown in Fig. 2. In any given specimen the concentrations of glycine, histidine, glutamine, serine, threonine, and alanine are always the greatest relative to those of the other amino acids present. These are the amino acids which normally have the higher plasma concentrations and exhibit the higher endogenous renal clearances (Fig. 1). Indeed, only when their concentrations in the urine are grossly elevated do others begin to appear in appreciable amounts. Serial studies during resolution of severe hyperaminoaciduria in patients treated for vitamin D deficiency states, lead poisoning, and congenital renal tubular acidosis show that these proportionate relationships between individual amino acid concentrations are maintained as the over-all severity of the aminoaciduria decreases toward normal.[43, 44]

These observations suggest that the pattern of generalized renal hyperaminoaciduria results from a proportionate increase in the clearances of all

amino acids. They suggest that some aspect of renal tubular function common to the reabsorption of all amino acids is impaired in all of these diseases. The published data on the sipidus may occur either as discrete entities or as components of multifactorial tubular defects.[2, 3]

Aminoaciduria, usually of the renal type, is associated quite frequently

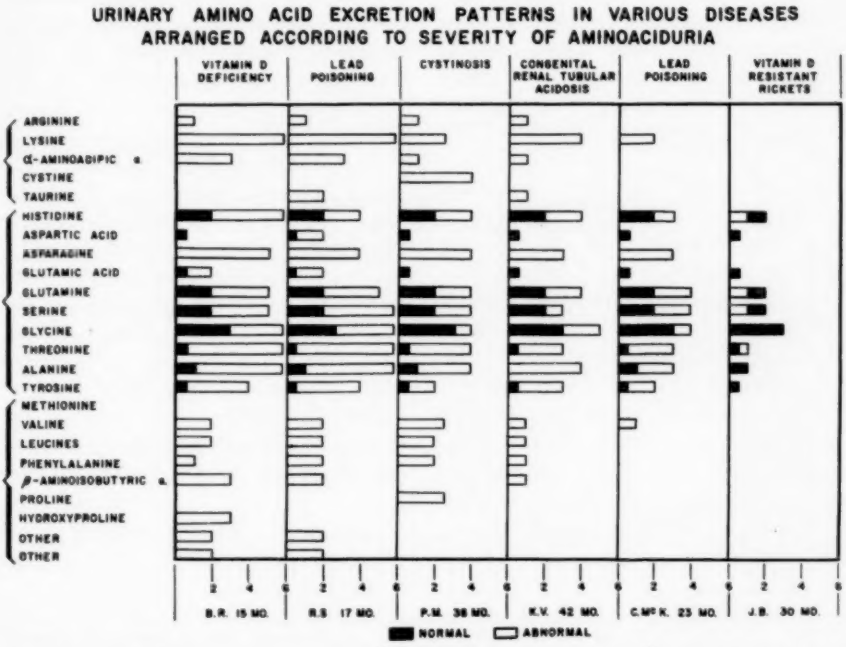

Fig. 2.—The data shown are based upon semiquantitative paper chromatograms of urine. The concentrations of each amino acid are presented graphically on an arbitrary scale of color intensity (scale is 1 to 6). The solid portions of the bars represent average color values from 10 normal children and are superimposed upon each patient's values. The urine samples are arranged from left to right in order of decreasing over-all severity of aminoaciduria. Reading from left to right, a proportionate decrease in relative amino acid concentrations occurs as over-all severity decreases. This illustrates the qualitative similarity between these patterns. The clinical diagnosis is shown at the top of each pattern.

diseases classified in Table I as exhibiting generalized renal aminoaciduria are in accord with this concept.

Generalized renal hyperaminoaciduria is frequently associated with other disorders of renal tubular function. Table II enumerates some of the combinations of renal tubular dysfunction which may occur simultaneously in one patient. For example, renal glycosuria, "potassium-losing nephritis," and nephrogenic diabetes in-

with hypophosphatemic states, which in turn are frequently associated with rickets or osteomalacia (Table II). Some of these hypophosphatemic states can be differentiated on the basis of the amino acid excretion pattern with which they are associated. For example, there is the specific pattern of "vitamin D resistant osteomalacia with hyperglycinuria" previously described.[4] In several untreated children with vitamin D resistant rickets (both

familial and nonfamilial), urinary amino acid excretion patterns have been normal as determined by paper chromatography in this laboratory (Fig. 2).[43] This confirms the report of Dent and Harris.[4] These patterns are readily distinguished from the pattern of generalized renal aminoaciduria found in vitamin D deficiency states,[45, 46] the Fanconi syndrome,[1, 13] and congenital renal tubular acidosis[44] in which quite different therapy is required. On the other hand, the renal aminoaciduria of vitamin D deficiency does not distinguish between deficiency due to impaired intestinal absorption (biliary obstruction and steatorrhea) and that due to deficient intake of vitamin D.

The term "Fanconi syndrome" is here used to signify the triad of hypophosphatemia with hyperphosphaturia, renal aminoaciduria, and renal glycosuria. This triad of renal tubular dysfunctions is seen in a variety of clinical syndromes of apparently diverse origin, both congenital and acquired. Its occurrence in these diverse disorders strongly supports the concept that the triad is produced by an effect upon some renal tubular mechanism common to the reabsorption of all of these substances. In the congenital forms of the Fanconi syndrome all components of the triad are usually obvious as is radiologically overt rickets. In the acquired forms the pattern is variable. In lead intoxication the complete syndrome including overt rickets has been encountered but once in a child whose plumbism was both chronic and

TABLE II. RENAL TUBULAR SYNDROMES

CLINICAL SYNDROME	RICKETS OR OSTEOMALACIA	SPECIFIC RENAL TUBULAR FUNCTIONS THAT MAY BE DERANGED					
		REABSORPTION OF				EXCHANGE OF	
		PHOSPHATE	AMINO ACIDS†	GLUCOSE	WATER	POTASSIUM	HYDROGEN
Vitamin D resistant rickets*	+	+	–	–	–	–	–
Vitamin D deficiency states	+	+	+	–	–	–	–
Fanconi syndrome congenital (including cystinosis)	+	+	+	+	±	±	±
acquired (lead intoxication, Wilson's disease)	±	±	±	±	–	–	–
Luder-Sheldon syndrome	–	–	+	+	–	–	–
Renal glycosuria	–	–	–	+	–	–	–
Nephrogenic diabetes insipidus	–	–	–	–	+	–	–
"Potassium-losing nephritis"	–	–	–	–	±	+	–
Congenital renal tubular acidosis	+	+	+	±	+	±	+

This table is offered not as complete listing but to illustrate the various multifactorial tubular defects which may be present in a patient. For example, essential cystinuria, a specific renal aminoaciduria, is omitted. In both the congenital and acquired forms of the Fanconi syndrome variations from the pattern shown may be encountered. Note the frequency with which generalized renal aminoaciduria is associated with both hypophosphatemia and rickets or osteomalacia. Skeletal abnormalities often constitute the presenting clinical complaints in these syndromes.

*Vitamin D resistant osteomalacia with hyperglycinuria and some cases with a more generalized increase in amino acid excretion have been reported.

†Refers to generalized renal aminoaciduria.

severe.[47] The triad of hypophosphatemia, aminoaciduria, and glycosuria without obvious rickets has been found in a number of acute and severe cases of childhood lead poisoning.[43] In less severe cases only hyperaminoaciduria is found.[43, 48] Hyperaminoaciduria alone has been reported in adults exposed to lead and other heavy metals.[49] In Wilson's disease hyperaminoaciduria is a frequent but inconstant finding and osteomalacia a rare one.[50, 51] In galactosemia[52] hyperaminoaciduria is reversible and occurs only when galactose is present in the diet. Other components of the Fanconi syndrome have not been reported in galactosemia. The reversibility and quantitative variability of hyperaminoaciduria in these diseases suggest that generalized renal hyperaminoaciduria is a very sensitive but nonspecific indicator of impaired renal tubular function.

Generalized renal hyperaminoaciduria may represent one component of multifactorial tubular defects; it may also represent but one aspect of widespread systemic disease. The aminoaciduria of galactosemia, of Wilson's disease, and of heavy metal intoxication results from renal injury; yet, the most striking clinical features of these diseases usually point to hepatic or central nervous system involvement. Pellagrous skin rash, cerebellar ataxia, and dementia are the striking features of the Hartnup syndrome, although its aminoaciduria is renal in origin. The manifold expressions of these diseases indicate widespread defects involving many organs.

Aminoacidurias of the "overflow" type indicate predominately extrarenal disease. In phenylketonuria, argininosuccinicaciduria, and "maple syrup urine disease,"[53, 54] defective cerebral development dominates the clinical picture. Clinically, they may be indistinguishable; yet, each is associated with a specific pattern of aminoaciduria. Still more complex clinical and metabolic problems may be encountered. Recently, Smith and Strang[55] reported a case which in certain respects resembled phenylketonuria. Clinically, the infant exhibited cerebral defect and recurrent bouts of edema. The urine contained, in addition to phenylpyruvic acid, α-hydroxy-butyric acid. Urinary amino acid chromatograms revealed, in addition to phenylalanine, excessive amounts of tyrosine and methionine which served to distinguish the pattern of aminoaciduria in this syndrome from that seen in phenylketonuria (excessive phenylalanine only). When effective therapies for these disorders are developed, accurate diagnosis will become mandatory. Urinary amino acid excretion patterns can be of considerable aid in differentiating these syndromes.

Similarly, in clinical research amino acid studies have been of value in delineating metabolic aberrations and genetic patterns. Argininosuccinicaciduria and "maple syrup urine disease" are recently described forms of cerebral defect in which urinary amino acid studies provided the initial clue to the error of metabolism involved in each disease. Such studies may be expected to bear additional fruit in the future. Many of the syndromes discussed represent inborn errors of metabolism. The patterns of inheritance in cystinuria and β-aminoisobutyricaciduria, for example, have

been determined largely through urinary amino acid studies. Family studies of amino acid excretion represent a means whereby the clinician may often identify unsuspected cases in some of these genetic disorders.

In summary, urinary amino acid studies may provide valuable information for the clinician in the differential diagnosis of syndromes in which the presenting clinical features are nonspecific and point to skeletal, renal, hepatic, or cerebral disease.

SUMMARY

Hyperaminoaciduria is significant as a manifestation of aberrant cellular function in the kidney and in other organs (bone, liver, and brain in particular). The various patterns of hyperaminoaciduria may be described in terms of the endogenous renal clearances of the individual amino acids. Normally, those amino acids with the higher plasma concentrations or with the higher endogenous renal clearances or with both appear in the urine in the greater concentrations. The pattern of generalized renal aminoaciduria results when mechanisms common to the reabsorption of all amino acids are impaired. This pattern is nonspecific and may be seen in a variety of syndromes, the most common of which are the Fanconi syndrome and vitamin D deficiency states. Generalized renal hyperaminoaciduria may be distinguished from the generalized "overflow" aminoaciduria of severe hepatic cell damage and from the specific patterns of renal and systemic origin. A classification of aminoaciduria on this functional basis is presented.

The finding of generalized renal hyperaminoaciduria in diseases of varied etiology suggests that this type of aminoaciduria is a very sensitive manifestation of impaired renal tubular function. Although the aminoaciduria results from renal injury, defects involving many organs may be present in these diseases. Indeed, cerebral and hepatic features may predominate the clinical picture in some of these diseases (e.g., galactosemia, lead encephalopathy, and Wilson's disease).

The occurrence of the triad of renal aminoaciduria, glycosuria, and hypophosphatemia provides further evidence of an interrelationship between the renal tubular mechanisms for the reabsorption of these substances. Still other derangements of renal tubular function may be present when this triad is found. This emphasizes the need for complete studies of renal function in clinical problems in which any one discrete tubular function is obviously deranged.

Distinctive "patterns of aminoaciduria" are found in various forms of rickets and osteomalacia. As the therapy differs in each, urinary amino acid studies may be helpful in therapeutic management. Likewise, specific patterns of aminoaciduria facilitate the accurate diagnosis of some forms of cerebral defect which have similar clinical features (e.g., phenylketonuria and "maple syrup urine disease").

The study of patterns of aminoaciduria has proved to be a valuable technique in genetic surveys and in the unraveling of obscure metabolic disorders in which the metabolism of individual amino acids and closely related intermediates is involved. As the technique of paper chromatography becomes more widely used, it may be anticipated that still other errors of

intermediary metabolism will be identified among syndromes that are without distinctive clinical features.

Semiquantitative paper chromatographic estimation of urinary acid patterns is adequate for most clinical problems in which aminoaciduria is suspected.

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A ROLE FOR TOCOPHEROL IN HUMAN NUTRITION

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THIS paper will attempt to summarize evidence which we believe establishes a role for tocopherol in human nutrition.

A specific dietary fat-soluble factor, vitamin E, was reported necessary for reproduction in the rat over 35 years ago. Since then its importance to the health of numerous laboratory and domestic animals has led to extensive investigations of many pathologic states which result from dietary deficiency. These include reproductive failure, growth failure, nutritional muscular dystrophy, focal necrosis and fibrosis of cardiac muscle, deposition of acid-fast (ceroid) pigment in smooth muscle, subcutaneous collections of edema fluid, at times greenish because of decomposed hemoglobin, grossly hemorrhagic extravasations in embryos and fetuses, hemorrhagic necrosis of the liver, depigmentation of dental enamel, increased hemolysis of erythrocytes in dialuric acid or dilute hydrogen peroxide, increased intravascular hemolysis when large doses of vitamin K are administered, anemia, and granulocytosis. Increased urinary excretion of amino acids and allantoin, increased xanthine oxidase activity in the liver, increased incorporation of formate into the purines of ribonucleic and desoxyribonucleic acids of skeletal muscle and bone marrow have been reported for vitamin E deficient animals. The variety of findings has led to intense interest in the mechanism of action of tocopherol. Critical studies which have exploded claims of benefit for tocopherol in sterility, progressive muscular dystrophy, and heart disease in the human, and the dependence of some of the manifestations of deficiency on other dietary factors as, for example, unsaturated fatty acids, sulfur amino acids, protein, pyridoxine, vitamin A, or selenium have, however, discouraged many clinical and laboratory investigators.

PATIENTS WITH STEATORRHEA

Since tocopherol is a fat-soluble vitamin, one might expect manifestations to develop in patients, and particularly in infants and children, with steatorrhea. As a matter of fact, a peculiar myopathy was described in 2 adults with steatorrhea as early as 1908, and deposition of ceroid pigment in smooth muscle has been reported in adults with the malabsorption syndrome. Low plasma tocopherols have also been reported.

In infants and children with cystic fibrosis of the pancreas, celiac syndrome, and congenital biliary atresia,

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Filer and his co-workers[1] found low plasma tocopherol and flat absorption curves. This work has been extended and confirmed and pediatric subjects with steatorrhea regularly have been found to have not only low plasma tocopherol but an increased hemolysis of erythrocytes in dilute hydrogen peroxide.[2, 3] The latter, which was suggested by Rose and György[4] as a measure of tocopherol deficiency, has a fairly good negative correlation with plasma tocopherol concentrations after the newborn period.[3]

Since plasma concentrations are not wholly satisfactory criteria of deficiency, a search was made for other evidences of deficiency; these have been found. Infants and children with cystic fibrosis of the pancreas and congenital biliary atresia show excessive creatinuria, a manifestation of tocopherol deficiency in rabbits and monkeys; in the children with cystic fibrosis of the pancreas this creatinuria has been markedly diminished by oral administration of tocopheryl esters.[5] Woodruff[6] has reported similar findings in an adult with xanthomatous biliary cirrhosis. In 6 children studied the decrease in creatinuria has been accompanied by a decrease in plasma creatine,[7] indicating that it is a prerenal effect, and further studies are being made to determine whether it is due to diminished synthesis of creatine, increased deposition of creatine in muscle, or decreased extrusion from muscle.

The biochemical evidence of tocopherol deficiency has been supported by two pathologic findings. Focal lesions, consisting of necrosis, fragmentation of muscle fibers, and cellular infiltration, have been found in skeletal muscles of patients dying with cystic fibrosis of the pancreas and congenital biliary atresia.[8-10] Although these lesions are not specific for vitamin E deficiency, they are characteristic of the lesions seen in the skeletal muscles of animals with nutritional muscular dystrophy. In one patient[8] they were associated with rickets and squamous metaplasia of epithelium, manifestations of deficiency of vitamins D and A, respectively. They have also been noted in patients with kwashiorkor who have, among other findings, low concentrations of plasma tocopherol.[11]

The second finding, ceroid pigment, a product of auto-oxidation of unsaturated lipids[12] which also occurs in animals with vitamin E deficiency, has been reported in gastrointestinal muscle of patients dying with cystic fibrosis of the pancreas, particularly if they are over 2 years of age.[10] It was less frequent in patients who died with biliary atresia, presumably because of their earlier exitus.

The findings of low plasma tocopherol, increased hemolysis of erythrocytes in hydrogen peroxide, elevated urinary and plasma creatine, all of which were reversed by tocopherol administration, and the pathologic findings of focal lesions in skeletal muscle and ceroid pigment in gastrointestinal smooth muscle combine to establish that the biologic correlates of tocopherol deficiency do indeed occur in human subjects who fail to absorb vitamin E as part of their steatorrhea. Even though no clinical benefits of tocopherol administration have been shown,[13] the biochemical and pathologic findings

warrant routine administration of tocopheryl esters to patients with cystic fibrosis of the pancreas. Doses of 10 mg. per kilogram per day of a water-miscible ester, tocopheryl acetate, regularly raise the blood concentration to normal levels, but no studies have been made of the minimal effective dose, a matter of considerable practical import.

NEWBORN INFANTS

Newborn infants, full term and premature, are born with low plasma tocopherol concentrations which are 1/5 to 1/7 those of the mother at term[3] and with low body stores.[14] The feeding of breast milk to full-term infants produces a rapid rise in plasma tocopherol to normal adult levels. Artificial feeding also does so, but more slowly.[15, 16] In premature infants fed partially skimmed cow's milk mixtures plasma tocopherol levels remain low. In view of their defect in fat absorption the lower tocopherol content of stored as compared with fresh milk and the performance of full-term infants on whole cow's milk mixtures, the probabilities are that plasma tocopherol concentrations would remain low unless the diets were supplemented. Such supplementation has been recommended for prematurely born infants,[17] and tocopherol has been added to a powdered, partially skimmed cow's milk (Alacta) used extensively for feeding premature infants.[18] This supplementation is probably indicated. We have not yet recommended it but we do continue to search for additional indications for the need for tocopherol in this age group. In view of the length of time it takes some animals to develop gross evidence of deficiency and human adults to develop low plasma tocopherol on tocopherol deficient diets,[19] it may be that we are being excessively conservative. Popular, costly overuse of supplementary vitamins contributes to our stand.[20]

CONCLUSIONS

The findings of low plasma tocopherol, increased hemolysis of erythrocytes in dilute hydrogen peroxide, increased urinary and plasma creatine in patients with steatorrhea, and their reversal with tocopherol administration in patients with cystic fibrosis of the pancreas constitute definite evidence for the existence of a tocopherol deficiency in the human subject. This is supported by postmortem findings of focal lesions in the skeletal muscles and of ceroid pigment in gastrointestinal smooth muscle of patients with cystic fibrosis of the pancreas and congenital biliary atresia. Although these lesions are not specific, they are characteristic of tocopherol deficiency in animals. The findings of low plasma and body tocopherol and increased hemolysis of erythrocytes in newborn infants, both full term and premature, warrant continued study of these subjects for biologic correlates of these low stores of tocopherol.

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METABOLIC ABNORMALITIES OF ERYTHROCYTES FROM PATIENTS WITH CONGENITAL NONSPHEROCYTIC HEMOLYTIC ANEMIA

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IN RECENT years the mechanism of hemolysis in a variety of hemolytic disorders has been partially elucidated through studies on the biochemical characteristics of normal as well as abnormal erythrocytes. A noteworthy example of the fruitfulness of such an approach was the demonstration by Alving and his associates of the altered metabolism in red cells from individuals susceptible to the hemolytic effects of certain drugs and chemicals: primaquine, phenacetin, sulfanilamide, naphthalene, Furadantin, Gantrisin, Kynex, and others. This unique sensitivity to hemolysis was related to an "inborn error of red cell metabolism" which apparently is innocuous until persons with these erythrocytes are exposed to potentially hemolytic compounds. A comprehensive review of the role of various investigators in elucidating the mechanism and genetic transmission of drug-induced hemolysis was recently published by Beutler.[1] Drug-sensitive erythrocytes are not detectable by routine hematologic procedures such as examination of smears of the peripheral blood, the Coombs test, electrophoretic analysis of hemoglobin, and osmotic and mechanical fragility tests. The description of certain biochemical alterations in these red cells, however, has resulted in the development of relatively simple in vitro tests for their detection. Drug-sensitive erythrocytes have a slight deficiency of reduced glutathione (GSH) as well as a marked fall in GSH when whole blood is incubated with acetylphenylhydrazine (GSH stability test). Another abnormality is a reduction of glucose-6-phosphate dehydrogenase activity, the enzyme which initiates the hexose monophosphate shunt. As was predicted, these biochemical alterations are genetically determined and are probably transmitted by a sex-linked gene with variable expressivity. The racial distribution and intraracial frequency of these red cell defects have also been studied. Drug-sensitive erythrocytes have been described in American Negroes, Orientals, Greeks, Italians, and Sephardic Jews. In the first group approximately 15 per cent of the men and 2 per cent of the women manifest a marked deficiency of red cell glucose-6-phosphate dehydrogenase activity, while 5 per cent of the women have only a moderate decrease of enzyme activity. Men and women with a marked reduction of glucose-6-phosphate dehydrogenase activity have been designated "reactors," whereas those women with a partial decrease

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in red cell enzyme activity have been termed "intermediates." Intermediate degrees of the enzyme deficiency have not been found in Negro men.

The term "congenital nonspherocytic hemolytic anemia" has been applied to congenital and sometimes familial hemolytic disorders which are not associated with a characteristic alteration of red cell morphology.[2-8] The diagnosis is made, therefore, only after congenital hemolytic diseases identifiable by abnormally shaped erythrocytes have been excluded (Mediterranean anemia, hereditary spherocytosis, the hemoglobinopathies, and hereditary elliptocytosis). A review of the case reports of patients with "congenital nonspherocytic hemolytic anemia" reveals certain features which are common to all of them: the osmotic and mechanical fragility studies are either normal or only slightly increased; the life span of the patient's erythrocytes in an autologous or homologous environment is reduced; splenectomy is usually not beneficial; the disease appears to be transmitted as a Mendelian dominant. Other findings, however, indicate that not all of these patients have the same hemolytic disorder. In some the red cells are heavily stippled[6, 7] and in others the rate of autohemolysis in vitro is greatly accelerated.[6, 9] Hence, it seems likely that the term "congenital nonspherocytic hemolytic anemia" as it is currently used signifies a polyglot group of hemolytic conditions.

Very little is known about the nature of the biochemical defect which shortens the survival time of nonspherocytic erythrocytes. By studying the effect of glucose on autohemolysis, on the increase in osmotic fragility, and on cation changes of red cells incubated in vitro, Selwyn and Dacie[9] were able to distinguish two varieties of "congenital nonspherocytic hemolytic anemia." More recently Newton and Bass[10] reported an abnormality of glutathione metabolism and a total absence of glucose-6-phosphate dehydrogenase activity in red cells from three Italian children with nonspherocytic hemolytic anemia. Similar biochemical abnormalities were not demonstrable in the parents of these patients.

During the past 2 years we have observed 6 cases of congenital nonspherocytic hemolytic anemia from 5 unrelated families. One of these patients, a Caucasian boy, developed hemoglobinuria following ingestion of Phenacetin, and biochemical abnormalities comparable to those described in primaquine-sensitive individuals were demonstrated in his erythrocytes. Following this observation, similar studies were done on the other 5 patients, and in the present communication the results and significance of these findings are presented and discussed.

CASE REPORTS

CASE 1.—Patient E. R., a 6-year-old white boy, was referred to this hospital for investigation of a chronic hemolytic anemia. He has observed to be icteric 24 hours after delivery, but no AB-O or RH incompatibility was demonstrated. The jaundice disappeared in one week, and the patient was considered normal until 2 years of age. Because of pallor, his peripheral blood was examined, and the parents were told that he had an unusual type of hemolytic anemia. The patient remained asymptomatic, and periodic blood counts revealed that

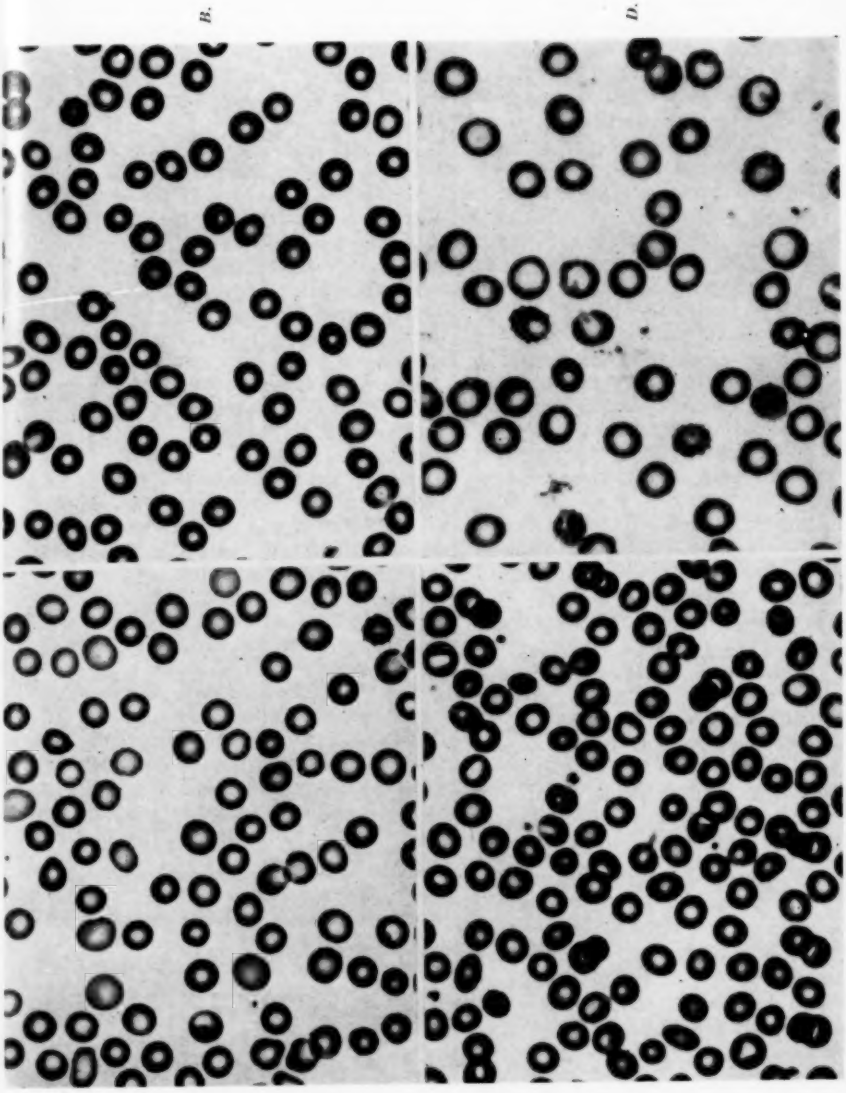

FIG. 1.—Smears of peripheral blood stained with Wright's stain (×750). *A*, Blood from Case 1 on Feb. 12, 1958. The red cells are normocytic and an occasional macrocyte is present. *B*, Blood from the maternal grandfather of Cases 1 and 2 on April 2, 1958. The red cells are normal. *C*, Blood from Case 3 on Nov. 24, 1958. The red cells are normal. *D*, Blood from Case 4 on Sept. 13, 1956. There are macrocytosis and slight hypochromia and some of the cells are target shaped. The nucleated red blood cell is a normoblast (reduction ⅙).

TABLE I. HEMATOLOGIC DATA ON PATIENTS WITH HEREDITARY NONSPHEROCYTIC HEMOLYTIC ANEMIA

SUBJECT	HEMOGLOBIN (GM. %)	HEMATOCRIT (%)	RETICULOCYTE COUNT (%)	WHITE BLOOD CELL COUNT (UNCORRECTED)	NUCLEATED RED BLOOD CELLS PER 100 WHITE BLOOD CELLS	PLATELETS	BILIRUBIN (MG. %) TOTAL	BILIRUBIN (MG. %) DIRECT	DIRECT COOMBS TEST	SPLENECTOMY (DATE OF)
Family R										
Father										
2/12/58		47.0	2.5	5,750	0	Normal				
Mother										
2/12/58		40.0	1.6	4,850	0	Normal				
Case 1										
2/12/58		34.0	10.0	5,750	0	Normal	0.58	0.12	Negative	
Brother										
2/12/58		37.0	0.8	8,700	0	Normal				
Case 2										
2/12/58		32.0	7.6	6,000	0	Normal	0.74	0.11	Negative	
Maternal grandmother										
4/3/58	13.0	42.5	2.5	7,000	0	Normal	1.4	0.1		
Maternal grandfather										
4/3/58	10.8	35.0	14.3	6,100	0	Normal	1.2	0.2	Negative	
Family K										
Father										
11/24/58	15.0	46.0	1.4		0	Normal	1.0	0.1		
Mother										
11/24/58	11.4	37.5	2.4		0	Normal	0.6	0.2		
Brother										
11/24/58	11.8	37.7	1.6		0	Normal	1.2	0.2		
Case 3										
11/24/58	11.2	33.5	10.8	4,300	0	Normal	2.0	0.2	Negative	
Case 4										
7/23/48	4.1	14.5	14.0	16,800	12	252,000	1.2	<0.8		3/7/49
8/5/49	5.9	22.0	37.8	32,500	81	660,000	1.0	<0.8		
9/13/56	6.1	23.9	46.2	13,750	10	Increased			Negative	
Case 5										
9/13/40	6.1	17.6	10.0	6,900	2	150,000	3.5	0.56		11/1/40
5/18/41	5.0		12.0	38,000	17	444,000	0.4	0.1		
7/27/54	9.3	30.0	24.0	8,500		Normal				
7/14/58	8.4	30.5	43.0	9,650	2	746,000	5.0	<0.8	Negative	
Family S										
Father	15.0	47.0	1.2		0	Normal				
Mother	12.4	38.9	1.6			Normal				
Case 6	13.4	41.6	0.5	8,800	0	Normal	0.6	0.2		Oct., 1947

the hemoglobin ranged between 10 and 12 Gm. per cent and the reticulocyte count between 6 and 10 per cent. At 5¾ years of age he developed an upper respiratory infection which was treated with one injection of Bicillin and 3 "fever tablets," each containing 2.5 Gr. of Phenacetin. One day later the patient was very pale, his sclerae were icteric, and he voided black urine. He was admitted to another hospital where the following laboratory data was learned: hemoglobin was 7.4 Gm. per cent; hematocrit, 27.0 per cent; white blood cell count, 5,800 per cubic millimeter; reticulocyte count, 4.7 per cent; direct Coombs test, negative; serologic studies for cold and acid hemolysins were negative; electrophoresis of hemoglobin, AA pattern; total bilirubin, 0.58 mg. per cent (direct was 0.12 mg. per cent); blood urea nitrogen, 7.2 mg. per cent; nonincubated osmotic fragility studies were normal. No intact red cells were seen in the urine, but the test for occult blood was positive. The color of the urine returned to normal over the next 2 days, and the patient was discharged on the tenth hospital day with a hemoglobin value of 10.6 Gm. per cent. Five weeks later the patient was evaluated at this hospital. The sclerae were white, the heart was not enlarged, and the liver and spleen were not palpable. Hematologic data are listed in Table I, and morphology of the erythrocytes is illustrated in Fig. 1.

CASE 2.—Ed. R., the brother of E. R. (Case 1), is 2½ years old and has been followed at another hospital because of a hemolytic anemia first observed at 1 year of age. The mother's pregnancy with this patient was normal and the patient had no clinical icterus during the neonatal period. At 11 months of age he was noted to be pale, and an examination of the peripheral blood several weeks later revealed a hemoglobin of 10 Gm. per cent and a reticulocyte count of 15 per cent. Subsequently, the patient's clinical condition and hemoglobin and reticulocyte values remained unchanged, and he continued to do well until 2 years of age. At this time an upper respiratory infection occurred which was treated with tetracycline, and although the fever and cough subsided, marked pallor ensued and he was hospitalized. The physical examination on admission was normal except for moderate pallor of the skin. The sclerae were not icteric and the liver and spleen were not palpable. The following laboratory studies were obtained: hematocrit was 27.0 per cent; reticulocyte count, 20.0 per cent; total white blood cell count, 20,700 per cubic millimeter; a differential count of the leukocytes showed 27 per cent polymorphonuclear neutrophils, 65 per cent lymphocytes, 7 per cent eosinophils, and 1 per cent basophils; direct Coombs test negative; paper electrophoresis of hemoglobin, hemoglobin AA; total serum bilirubin, 0.74 mg. per cent (direct was 0.11 mg. per cent); nonincubated osmotic fragility study, normal; urinalysis, normal. No specific therapy was given to the patient and he was discharged on the fifth hospital day.

When seen at this hospital 6 weeks later, the physical examination was normal. The hematologic data obtained on that visit are reported in Table I, and the morphology of the erythrocytes is described in Fig. 1. No Heinz bodies were seen in the red cells and the nonincubated and incubated osmotic fragilities were normal.

Family History (Cases E. R. and Ed. R.).—A partial pedigree of this family is outlined in Fig. 2. Both parents and the brother of these patients are living and well and no abnormalities were demonstrable in their peripheral blood. The maternal grandfather is 56 years old, and he has experienced episodes of jaundice since childhood which are usually associated with the passage of dark yellow urine. At 36 years of age extensive blood studies were done at another clinic, and following these the

patient was told that he had a "congenital nonspherocytic hemolytic anemia." A cholecystectomy was done at 40 years of age because of gallstones. Since that time the patient has not been aware of icterus, although there have been occasional episodes of fatigue and dyspnea. The most recent peripheral blood findings in this man are listed in Table I. There are 3 maternal greatuncles: one died at 40 years of age and the cause of death is unknown; the second died at 60 years of age while being treated for a hemolytic anemia; the third is 68 years old and is occasionally transfused because of a chronic hemolytic anemia.

The ancestral background of this family is Germanic and English, and the family pedigree has been traced through four generations.

CASE 3.—This 10-year-old white boy, patient A. K., was seen because of intermittent episodes of jaundice and anemia since birth. Both parents and an 8-year-old brother are living and well. A maternal uncle, 40 years of age, had transient icterus at the age of 9 and 18 years and during the second bout of jaundice was told that he had a hemolytic anemia. His general health since that time, however, has been excellent. The members of this family are American with mixed English, French, and Germanic antecedents.

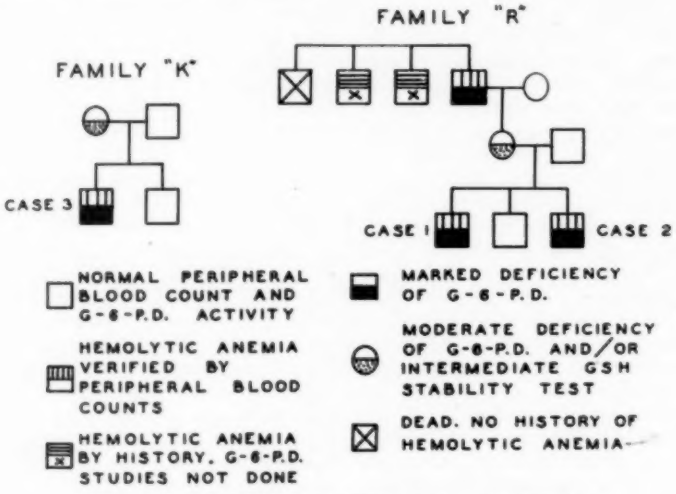
FAMILY "R"
FAMILY "K"
CASE 3
CASE 1
CASE 2
NORMAL PERIPHERAL BLOOD COUNT AND G-6-P.D. ACTIVITY
HEMOLYTIC ANEMIA VERIFIED BY PERIPHERAL BLOOD COUNTS
HEMOLYTIC ANEMIA BY HISTORY. G-6-P.D. STUDIES NOT DONE
MARKED DEFICIENCY OF G-6-P.D.
MODERATE DEFICIENCY OF G-6-P.D. AND/OR INTERMEDIATE GSH STABILITY TEST
DEAD. NO HISTORY OF HEMOLYTIC ANEMIA

Fig. 2.—Family pedigree of Cases 1 and 2 and Case 3.

The patient was first noted to be jaundiced at 2 months of age. The parents were told that their child was slightly anemic and an oral iron preparation was prescribed. The patient was asymptomatic until 5 years of age when jaundice recurred and persisted for a period of 3 months. Since that time there have been three episodes of jaundice, all preceded by an upper respiratory infection and all associated with severe anemia and passage of dark orange-colored urine. During two of these attacks he was hospitalized and transfused with whole blood. The patient has received short courses of penicillin, tetracycline, Aureomycin, and acetylsalicylic acid, but the onset of the icteric-anemia spells was not related to the administration of these drugs.

When seen at this hospital the patient was a well-developed and well-nourished boy. The sclerae were

white and the liver and spleen were not palpable. Hematologic data on the patient and his family are recorded in Table I; the appearance of the patient's erythrocytes is illustrated in Fig. 1. Nonincubated and incubated osmotic and mechanical fragility studies were normal.

CASE 4.—Patient R. J. is a 12-year-old American Negro girl who was transferred to this hospital at 10 days of age because of anemia and jaundice. The patient's birth weight was 5 pounds, 9 ounces, and the perinatal course was uneventful until the fifth day of life. At this time skin and scleral icterus appeared, the edge of the spleen was felt 4 cm. below the costal margin, and the hemoglobin was 5 Gm. per cent. A transfusion of 18 ml. of whole blood was given into the superior sagittal sinus. Following this procedure the icterus deepened and the patient was transferred to this hospital.

On admission the patient was pale, listless, and intensely jaundiced. The edges of the liver and spleen were palpable 3 cm. below the costal margin. Routine laboratory studies revealed: hemoglobin was 4.2 Gm. per cent; the total nucleated cell count was 11,000 per cubic millimeter; the differential count showed 50 per cent polymorphonuclear neutrophils, 42 per cent lymphocytes, 7 per cent monocytes, with 8 nucleated erythrocytes per 100 leukocytes; three blood cultures were sterile; urinalysis was normal; the total bilirubin was 16.8 mg. per cent (direct, 1.2 mg. per cent); the serologic test for syphilis and the direct Combs test were negative; the patient's blood group was O M N Rh_1; no circulating Rh antibodies were demonstrable in the mother's serum. Paper electrophoresis of hemoglobin

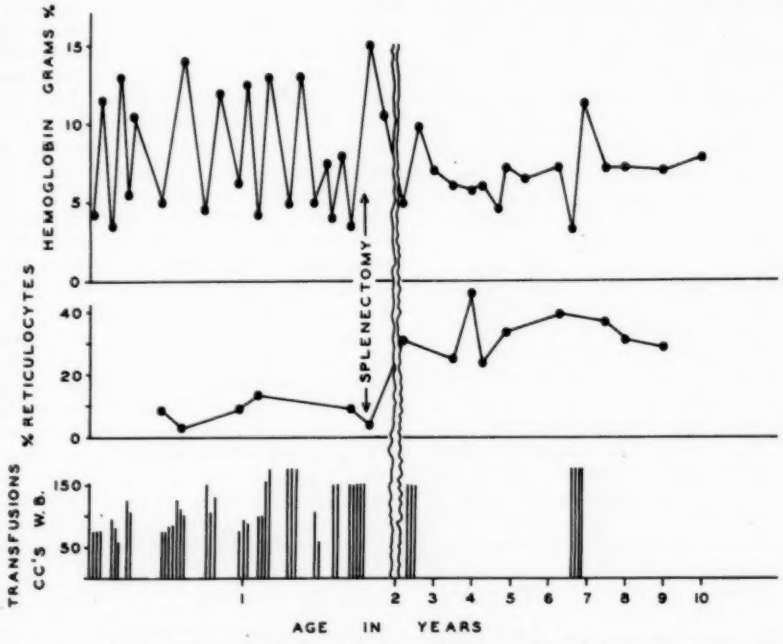

Fig. 3.—Variations of hemoglobin and reticulocyte values and frequency of transfusions in Case 4. Splenectomy was performed at 1 10/12 years of age.

done when the patient was 10 years of age revealed only hemoglobin A. The nonincubated and incubated osmotic and mechanical fragility tests were each normal when the patient was 8 and 10 years of age, respectively.

As shown in Fig. 3 the patient was regularly transfused every 4 to 6 weeks during the first 2½ years of her life. Following splenectomy at 3 years of age the recticulocyte count rose to a range of 20 to 40 per cent, the hemoglobin stabilized at a level of 8 Gm. per cent, and only two transfusions have been given in a period of 9 years since the operation. Detailed hematologic data before and after splenectomy are listed in Table I, and morphology of the erythrocytes is depicted in Fig. 1.

The parents, American Negroes, are living and well and their peripheral blood findings are normal.

CASE 5.—Patient R. C., a 19-year-old white boy, was first seen at 1½ years of age, with a history of jaundice and recurrent anemia from 2 days of age. The parents, two brothers, and one sister are living and well. Peripheral blood findings in the parents are normal. The mother's pregnancy with this patient and the delivery were normal. Icterus of the skin and sclerae was observed at 2 days of age. The intensity of the jaundice lessened and the baby was well until 7 weeks of age. During an upper respiratory infection marked pallor was observed and he was transfused with whole blood. Two subsequent transfusions were given at 3 and 16 months of age, and the patient was referred to this hospital for further studies at 18 months of age. At this time the skin and conjunctivae were pale, the sclerae were slightly icteric, the frontal and parietal bones of the skull were prominent, and the edges of the liver and spleen were felt 3 cm. below the costal margin. Admission laboratory findings revealed: hemoglobin was 5.4 Gm. per cent; the leukocyte count was 8,600; the differential count showed 37 per cent polymorphonuclear neutrophils, 6 per cent juvenile neutrophils, 49 per cent lymphocytes, 5 per cent monocytes, 2 per cent eosinophils, and 1 per cent basophils. There were 2 nucleated erythrocytes per 100 leukocytes, and the reticulocyte count was 10 per cent. Urinalysis was normal. The total bilirubin was 3.7 mg. per cent (direct, 0.9 mg. per cent). While in the hospital the patient developed an upper respiratory infection, and a pneumococcus, type VI, was cultured from the nasopharynx; sulfapyridine was given by mouth for a period of 10 days. One week later the patient was given two transfusions of whole blood and 24 hours later a splenectomy was performed. At the time of discharge, 3 weeks later, the hemoglobin was 10.1 Gm. per cent and the reticulocyte count was 3.5 per cent. Since splenectomy the patient has received only one transfusion. Slight pallor and scleral icterus have persisted, and the color of the urine becomes dark yellow during infections. Detailed hematologic data obtained before and after splenectomy are recorded in Table I. A total of 5 osmotic fragility studies, 2 done on nonincubated and incubated whole blood, were normal. Two nonincubated and incubated mechanical fragility tests were normal. The appearance of the erythrocytes in smears of the peripheral blood is shown in Fig. 1.

CASE 6.—This 15-year-old white girl has been extensively studied by Lange and Akeroyd[11] and was recently reported as a case of "congenital inclusion body hemolytic anemia with abnormal pigment metabolism." In résumé, patient "K. S." has had a hemolytic anemia associated with the passage of dark urine since 2½ years of age. She required transfusions of whole blood every 2 to 3 months until the age of 10 years, after which time no further transfusions were necessary. The father's blood was used for many of these transfusions since the administration of serologic compatible blood

from other donors was sometimes associated with severe hemolytic reactions. Other noteworthy features of this case are the recurrent upper respiratory infections, the occurrence of which has been unaffected by two tonsillectomies and adenoidectomies; the persistent petit mal and grand mal seizures which have been less frequent since the menarche at 11 years of age; the presence of inclusion bodies in approximately 14 per cent of the red blood cells; fasting blood sugars which have ranged between 27.8 and 54.0 mg. per cent. The patient has received Mesantoin, Tridione, Dilantin, and phenobarbital for her epileptic seizures and at present she is receiving Dilantin.

Detailed hematologic data as well as a description of the inclusion bodies of the erythrocytes, red cell survival studies, in vitro tests for Heinz body formation with phenylhydrazine, and characterization of the urinary pigment are reported in the article by Lange and Akeroyd.[11]

The current peripheral blood findings in the patient and her parents are listed in Table I. The ancestry of this family is English, Irish, and American Indian and there is no historical evidence of a hemolytic anemia in other members of the family pedigree.

METHODS*

Routine hematologic techniques as well as osmotic and mechanical fragility studies were performed according to methods described by Ham.[12] Blood for these procedures was obtained either by finger or venipuncture.

The amount of reduced glutathione (GSH) in whole blood was determined by the method of Grunert and Phillips as modified by Beutler and associates.[13] Values of GSH in venous blood obtained from 130 normal white adult men and women ranged between 53 and 84 mg. per 100 ml. of erythrocytes.

The stability of GSH in red blood cells was determined by the method of Beutler.[14] Whole blood, 1.0 ml., was incubated with 5.0 mg. of acetylphenylhydrazine (APH) for 2 hours at 37° C., and the concentration of GSH was assayed before and after the period of incubation. In some of the determinations sufficient glucose was added to whole blood to give a final concentration of added glucose of 200 mg. per cent. Three characteristic patterns of response have been described for this method. In normal adults the postincubation concentration of GSH in erythrocytes is greater than 40 mg. per 100 ml. of red blood cells; in approximately 5 per cent of Negro females the values vary between 22 and 40 mg. per 100 ml. of erythrocytes; in 14 per cent of Negro males and 2 per cent of Negro females levels of GSH are less than 20 mg. per 100 ml. of red blood cells.[15] Those individuals with values of GSH between 22 and 40 mg. per 100 ml. of erythrocytes have been designated "intermediate reactors," whereas persons with concentrations of GSH less than 20 mg. per 100 ml. of red blood cells have been termed "reactors." On the basis of family studies now in progress it has been determined that some of the "intermediate reactors" have postincubation GSH values as high as 45 mg. per 100 ml. of erythrocytes so that there is an overlap in the values of normal and "intermediate reactors."

*For the sake of brevity, the following abbreviations are used in the text:

APH = acetylphenylhydrazine,
G-6-P = glucose-6-phosphate,
G-6-P.D. = glucose-6-phosphate dehydrogenase,
GSH = reduced glutathione,
6-PG = 6-phosphogluconate,
RU-5-P = ribulose-5-phosphate,
TPN = triphosphopyridine nucleotide,
TPNH = reduced triphosphopyridine nucleotide.

Activity of glucose-6-phosphate dehydrogenase (G-6-P.D.) in the erythrocyte was determined by two methods. The first of these was a modification of one reported by Glaser and Brown[16] and has been described in a previous communication[17]; the second was patterned after the method of Glock and McLean.[18] In the first method the change in optical density at 340 mμ in a Beckman model DU spectrophotometer is followed every minute between 5 and 15 minutes after the addition of triphosphopyridine nucleotide (TPN) to a mixture of glucose-6-phosphate (G-6-P) and hemolysate. One unit of enzyme is defined as that amount which produces an optical density change at 340 mμ of 2.07 per minute per 3.0 ml. of reaction mixture, an optical density change which represents the reduction of 1 μm of TPN.

$$\text{(1) G-6-P} + \text{TPN}^{+} \xrightarrow[\text{dehydrogenase}]{\text{glucose-6-phosphate}} \text{6-phosphogluconolactone} + \text{TPNH} + \text{H}^{+}$$

$$\text{(2) 6-phosphogluconolactone} \xrightarrow{\text{lactonase}} \text{6-PG (6-phosphogluconate)}$$

$$\text{(3) 6-PG} + \text{TPN}^{+} \xrightarrow[\text{dehydrogenase}]{\text{6-phosphogluconic}} \text{RU-5-P (ribulose-5-phosphate)} + \text{CO}_2 + \text{TPNH} + \text{H}^{+}$$

Sum of (1), (2), and (3):

$$\text{G-6-P} + 2\ \text{TPN}^{+} \text{ ---------- } \text{RU-5-P} + \text{CO}_2 + 2\ \text{TPNH} + 2\ \text{H}^{+}$$

The rationale for the second method is based on the following observations. For each mole of G-6-P oxidized during the first three steps of the hexose monophosphate shunt, 2 moles of reduced triphosphopyridine nucleotide (TPNH) can be formed.

TABLE II. COMPARISON OF ACTIVITIES OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE

SUBJECTS	NUMBER	GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY (U./100 ML. OF ERYTHROCYTES)			
		GLUCOSE-6-PHOSPHATE AS SUBSTRATE		"DERIVED"	
		RANGE	AVERAGE	RANGE	AVERAGE
Normal white adult men and women	21	180-278	219	135-186	155
Negro women intermediate reactors	13	91-198	159	59-123	82
Men reactors					
Greek	1	6		0	
Sephardic Jew	1	1		0	
American Negro	5	6-56	30	2-24	13
Negro women reactors	5	22-95	62	6-44	27

These activities are determined by two different assay techniques in erythrocytes from normal white adults, Negro women "intermediate reactors," and men and women "reactors" of three racial groups.

All of the enzymes required for these reactions are present in normal hemolysates. Therefore, any assay technique for G-6-P.D. activity using G-6-P as substrate and quantified by rate of TPNH formation may give falsely

high values. In order to measure only G-6-P.D. activity Glock and McLean[18] have devised the following method. Rate of TPNH formation is measured at pH 8.0 in one cuvette with 6-PG as substrate (0.1 ml. of a 0.02 M. solution), and in the other cuvette with G-6-P and 6-PG as substrates (0.1 ml. of 0.02 M. solution of each). G-6-P.D. activity is calculated by substracting the value of the former from that of the latter, and in the data to be presented this value has been designated the "derived" G-6-P.D. value.

Data on enzyme activity as measured by these two methods are listed in Table II. These results were obtained on four groups of individuals: normal white adult men and women, Negro women who are "intermediate reactors," a group of men "reactors" (one Greek, one Sephardic Jew, and the rest American Negroes), and a group of Negro women "reactors."

RESULTS

Clinical groupings and Hematologic Data.—The clinical features and peripheral blood findings in these patients suggest that they can be divided into three groups. Cases 1, 2, and 3 comprise the first group; all are Caucasian boys. In these patients there was a mild to moderate reduction of hemoglobin values, persistent reticulocytosis, and minimal alteration of red cell morphology, with slight macrocytosis and polychromatophilia. The anemia occasionally exacerbated following upper respiratory infections and during some of these episodes was so severe that transfusions were necessary. Another significant clinical event in this group was the occurrence of hemoglobinuria in Case 1 after the administration of Phenacetin during an upper respiratory infection. Splenectomy has not been performed in any of these patients.

The second clinical group consists of Case 4, an American Negro girl, and Case 5, a Caucasian boy. In these patients anemia became apparent soon after birth, and frequent transfusions were required to maintain hemoglobin values above 5 Gm. per cent (see Fig. 3). The majority of the erythrocytes in smears of the peripheral blood were macrocytic and slightly hypochromic, some were target shaped, and a few were finely stippled. The reticulocyte counts were moderately elevated and nucleated red cells were always present in the peripheral blood. Following splenectomy in these two patients there was a marked rise in the reticulocyte count and, although anemia persisted, frequent transfusions were no longer necessary.

Case 6, a 15-year-old Caucasian girl, illustrates the third variety of congenital hemolytic anemia surveyed in this study. This patient experienced severe hemolysis between 2½ and 12 years of age and transfusions were required at 6 to 8 week intervals. Her course was complicated by frequent convulsive seizures which were only partially controlled by antiepileptic therapy. Also, fasting blood sugars in the range of 27 to 54 mg. per cent were observed. The red cells showed marked anisocytosis, poikilocytosis, stippling, and polychromatophilia, and in addition contained inclusion bodies which were demonstrable by the Leishman-Giemsa stain and in wet films of the peripheral blood. An abnormality of pigment metabolism was suggested because of the presence in

the urine of a substance belonging to the bilifuscin and mesobilifuscin groups. Splenectomy at 4 years of age had only a transient beneficial effect on the hemolytic process.

Glutathione Studies and Assays for Glucose-6-Phosphate Dehydrogenase Activity in the Erythrocytes.—The results of these studies are listed in Table III. Cases 1, 2, and 3 manifested a moderate to marked deficiency of reduced GSH in their erythrocytes, an abnormal decrease in GSH after incubation of whole blood with APH, and a complete deficiency of G-6-P.D. of the erythrocytes. In Cases 1 and 3 no reduction of TPN occurred in a 30 minute period even though the amount of hemolysate usually employed was doubled. The addition of normal hemolysate to the assay system at this time was followed by a normal rate of reduction of TPN.

GSH values of whole blood and results of the glutathione stability test were normal in Cases 4 and 5. G-6-P.D. activity determined by using G-6-P as substrate was elevated. "Derived" G-6-P.D. values, however, were normal.

Low whole blood GSH values were obtained in Case 6 and there was an abnormal decrease in GSH during the GSH stability test. When glucose was added to blood prior to the GSH stability test, the per cent of decrease of GSH was less but still greater than that observed in normals. G-6-P.D. activity as measured by both assay techniques was markedly elevated.

Family Studies.—Peripheral blood findings and the results of glutathione and enzyme studies on the families of the patients are presented in Tables I and III.

Cases 1 and 2: The parents of these patients as well as the brother and the maternal grandmother had no abnormalities of their peripheral blood. The maternal grandfather has had intermittent jaundice since childhood, and a cholecystectomy was performed at 40 years of age because of gallstones. When studied in this laboratory, his hematocrit was 35 per cent, the reticulocyte count was 14.3 per cent, and the morphology of the red cells was similar to that observed in Cases 1 and 2. Two brothers of the maternal grandfather also had a history of a chronic hemolytic anemia, but their blood was not available for study.

Whole blood GSH values, the glutathione stability determinations, and assays for G-6-P.D. activity were normal in the father, the brother, and the maternal grandmother. Results of these studies on blood from the maternal grandfather were comparable to those observed in the patients. The mother's glutathione stability test was in the intermediate range, and her G-6-P.D. value as determined with G-6-P as substrate was low normal (Table III).

Case 3: Peripheral blood findings in the parents and the brother of this patient were normal. Glutathione studies on the mother's blood revealed a normal level of GSH in her erythrocytes. After incubating blood with APH, however, the resulting decrease in GSH was similar to that observed in Negro women who are "intermediate reactors." The "derived" G-6-P.D. value was definitely decreased, although G-6-P.D. activity measured with G-6-P as substrate was low normal (Table III).

TABLE III. GLUTATHIONE VALUES OF THE WHOLE BLOOD, RESULTS OF THE GLUTATHIONE STABILITY TEST, AND LEVELS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY OF ERYTHROCYTES IN PATIENTS WITH CONGENITAL NONSPHEROCYTIC HEMOLYTIC ANEMIA

SUBJECT	REDUCED GLUTATHIONE (MG./100 ML. RED BLOOD CELLS)			ENZYME ACTIVITY (U./100 ML. RED BLOOD CELLS)	
	BEFORE INCUBATION WITH APH	AFTER INCUBATION WITH APH	GLUCOSE ADDED BEFORE INCUBATION WITH APH	GLUCOSE-6-PHOSPHATE DEHYDROGENASE (GLUCOSE-6-PHOSPHATE AS SUBSTRATE)	"DERIVED" GLUCOSE-6-PHOSPHATE DEHYDROGENASE
Family R					
Father					
2/12/58	60	59	64	198	
4/25/59				222	146
Mother					
2/12/58	57	41	39	185	
4/25/59				172	115
Case 1					
2/12/58	45	8		0	
4/25/59				0*	0
Brother					
2/12/58	62	53		226	
Case 2					
2/12/58				0	
Maternal grandfather					
4/3/58	45	14		0	
3/31/59				0*	1
Maternal grandmother					
4/3/58	98	81			
3/31/59				256	162
Family K					
Father					
11/25/58	70	49	51	258	162
4/3/59	66		59	224	132
Mother					
11/25/58	65	31	36	180	77
4/3/59	62		44	166	108
Brother					
11/25/58	56	42	44	272	158
Case 3					
11/25/58	23	1	0	0	0
4/3/59	28		7	0*	0
Case 4					
7/15/58	94	71	69		
7/16/58	64	50			
7/23/58	65	53			
7/25/58	77	66		300	152
8/1/58	67	48			
Case 5					
7/24/58	64	53		356	188
Family S					
Father					
3/17/59	41	28	27	337	240
3/27/59	45	28	31	314	215
Mother					
3/17/59	68	45	49	210	151
3/27/59	74	51	55	214	163
Case 6					
3/17/59	49	20	35	411	278
3/27/59	50	27	34	394	239

*Optical density changes were followed for a period of 30 minutes in these hemolysates.

Cases 4 and 5: Examination of the peripheral blood as well as nonincubated and incubated osmotic fragility studies on the parents of these patients were normal. No abnormalities of glutathione metabolism or enzyme activity were observed in erythrocytes from the mother of Case 4.

Case 6: Values for the hematocrit, hemoglobin, and reticulocyte count in the parents were normal. Wet films of the peripheral blood were examined for inclusion bodies, but none were seen. Glutathione and enzyme studies on the mother's blood were normal. The father had an abnormally low concentration of GSH before and after incubation of whole blood with APH. Activity of G-6-P.D. in his erythrocytes was markedly increased, although the magnitude of elevation was not as great as that observed in the erythrocytes of his daughter.

DISCUSSION

Most of the congenital hemolytic diseases can be classified by alerations in the morphology of the erythrocytes, the electrophoretic pattern of the hemoglobin, and the osmotic fragility of the red cells. There still remains, however, a small number of congenital hemolytic disorders which elude a clear-cut definition by these methods, and to this group has been applied the term "congenital nonspherocytic hemolytic anemia." Since the diagnosis is made only after certain well-recognized hemolytic disorders have been eliminated, one would expect to find a heterogeneous collection of anemias in the literature under this title. On the basis of differences in red cell morphology, severity of the hemolytic process, and response to splenectomy, some investigators have indeed suggested that not all patients with this diagnosis are suffering from the same disease.[2, 8] Attempts to elucidate the abnormality of red cell metabolism in these patients have also led to the recognition of several different forms. Selwyn and Dacie[9] described two types after observing changes in cation concentrations, rate of autohemolysis, and osmotic fragility of erythrocytes incubated in vitro. In Type 1, autohemolysis, osmotic, and cation changes on incubation were normal, but the degree of autohemolysis after the addition of glucose was decreased to a lesser extent than that observed in normal blood. In Type 2, the rate of autohemolysis, increase in osmotic fragility, and loss of potassium were markedly increased, and the addition of glucose had no effect on these abnormalities. Furthermore, erythrocytes from these patients utilized glucose at a rate which was 25 to 30 per cent of that observed in normals. Studies on phosphate metabolism of red cells from 4 patients with congenital nonspherocytic hemolytic anemia were performed by Prankerd.[19] Two groups were discernible: in one (3 patients) the concentration of adenosine triphosphate, the exchange of ^{32}P-orthophosphate, and the utilization of glucose were decreased; in the other (1 patient) these metabolic parameters were normal.

More recently, another type of metabolic abnormality has been described in erythrocytes from patients with chronic nonspherocytic hemolytic anemia. Newton and Bass[10] found an instability of glutathione and a deficiency of glucose-6-phosphate dehydrogenase (G-6-P.D.) in red cells of

a 4-year-old Italian boy who had a chronic nonspherocytic hemolytic anemia. Another publication by Newton and Frajola[20] reported similar findings on 2 other children, both Caucasian boys, who also were affected with this disease. A familial background for these abnormalities was not demonstrable.

In the present study a heterogeneous group of 6 patients with congenital nonspherocytic hemolytic anemia could be divided into three categories through glutathione and enzyme studies on the erythrocytes. The first group, comprising Cases 1, 2 and 3, manifested a slight to moderate decrease of whole blood GSH, an instability of GSH after incubation of blood with APH, and a deficiency of erythrocyte G-6-P.D. In the second group, Cases 4 and 5, these studies were normal; while in the third group, Case 6, the GSH values before and after exposure of blood to APH were moderately decreased, whereas the activity of G-6-P.D. was greatly increased. Severity of hemolysis and morphology of the red cells also differed in these three groups. In Group 1 the anemia and reticulocytosis were mild, although exacerbation of hemolysis occasionally occurred during infections. The red cells of these patients were normocytic and normochromic. The anemia and degree of reticulocytosis were more marked in Group 2; the erythrocytes were macrocytic, slightly hypochromic, and peripheral erythroblastemia was usually present, even prior to splenectomy. The one case in Group 3 was unique, for the clinical and hematologic data were quite unlike those found in other varieties of congenital hemolytic anemia.

Since all of these patients have a congenital disorder, one would anticipate that the anomalies of their erythrocytes are genetically determined. Routine hematologic procedures revealed no abnormalities in the peripheral blood from the parents of the patients in Group 1. Glutathione and enzyme values in the mother of Cases 1 and 2 and the mother of Case 3, however, were abnormal and were comparable to those observed in Negro women who are "intermediate reactors." Furthermore, the maternal grandfather of Cases 1 and 2 had a chronic nonspherocytic hemolytic anemia, and his glutathione and enzyme studies were abnormal (see Fig. 2). These findings suggest that the gene or genes responsible for this variety of nonspherocytic anemia are sex-linked and also indicate that the biochemical alterations of the red cell are a more sensitive index of gene action. In fact, the presence of the abnormal gene in the heterozygote woman would be overlooked if routine hematologic procedures were employed as a measure of its phenotypic expression and the gene would be considered impenetrant. Another example of the greater sensitivity of the biochemical method in demonstrating the genetic alteration is illustrated in the family of Case 6. Even though the morphology of the red cells and the hematocrit and reticulocyte values were normal in the parents, the father's glutathione and enzyme levels were similar to those observed in the patient.

The pathogenetic significance of these metabolic abnormalities has not

been established. The findings in the first group of patients were comparable in some ways to those observed in people with drug-sensitive erythrocytes. The morphology and fragility of the red cells were unaltered and the genetic transmission of the defect appeared to be the same. Unlike primaquine-sensitive individuals, the hemolytic process in these patients was continuous and not related to the administration of drugs, although in Case 1 hemolysis was accentuated during the administration of Phenacetin. Also, the patients were members of racial groups in which the phenomenon of primaquine-sensitivity has never been described, and their glutathione and enzyme deficiencies were occasionally more pronounced. The level of whole blood GSH in Case 3 and G-6-P.D. activity in Cases 1, 2, and 3 were less than that observed in most primaquine-sensitive individuals. When these enzyme values are compared to those of a person undergoing a primaquine-induced hemolysis, the difference becomes more marked. During the course of hemolysis in sensitive individuals, G-6-P.D. activity rises concomittantly with the reticulocyte count.[21] In patients with this variety of congenital nonspherocytic hemolytic anemia, enzyme activity is completely absent in the face of persistent reticulocytosis. It is suggested, therefore, that the mutant gene responsible for the enzyme deficiency in these patients differs from the one occurring in "primaquine-sensitive" people. Even though G-6-P.D. activity is not demonstrable, the possibility still exists that this abnormality is not the sole etiologic lesion and that additional genetic or environmental factors are necessary for the evolution of the disease. For example, the rate of hemolysis in these patients is increased during infections and following the administration of certain drugs.[20] Avoidance of these as well as other unrecognized nongenetic factors may lessen the degree of hemolysis.

Glutathione and enzyme studies on the patients in Group 2 were normal. Other methods are obviously necessary to demonstrate the inborn error of metabolism in the red cells of these patients. The changes observed in Case 6 and her father are quite unusual and, unlike the pattern observed in primaquine-sensitive persons, low GSH values are associated with a marked increase in G-6-P.D. activity. The results of the GSH stability test in this case indicate the importance of adding glucose during the performance of this procedure. In the absence of glucose, GSH values were 20 and 27 mg. per 100 ml. red blood cells after incubating blood with APH; with added glucose, these levels were 35 and 34 mg. per 100 ml. red blood cells. These findings are probably related to the hypoglycemia which the patient has demonstrated in the past and suggest that the red cell changes may be a manifestation of a generalized abnormality of carbohydrate metabolism.

SUMMARY

The clinical course, hematologic data, and red cell glutathione and glucose-6-phosphate dehydrogenase studies are reported for 6 patients who had a congenital nonspherocytic hemolytic anemia. On the basis of these findings the patients could be divided into three groups.

Group 1 consists of three Caucasian boys belonging to two families. The anemia and reticulocytosis were mild, red cell morphology was normal, red cell glutathione was unstable in the presence of acetylphenylhydrazine, and glucose-6-phosphate dehydrogenase activity was absent. Glutathione and enzyme studies on the family members indicated that these abnormalities were genetically determined and that their transmission was sex-linked.

A Caucasian boy and a Negro girl comprise Group 2. The anemia and reticulocytosis were quite marked, and the erythrocytes were macrocytic, slightly hypochromic, and occasionally target shaped and finely stippled. Nucleated red cells were usually present in the peripheral blood. Glutathione and enzyme studies were normal.

Group 3 consists of a 15-year-old Caucasian girl who was first described by Lange and Akeroyd.[11] She had a severe hemolytic anemia between 2½ and 10 years of age, and inclusion bodies were observed in the red cells. Frequent epileptic seizures and black-colored urine were also noted. Whole blood glutathione values and the glutathione stability test were abnormal. Erythrocyte glucose-6-phosphate dehydrogenase activity was increased. The father's peripheral blood count was normal, but his glutathione and enzyme values were similar to those observed in the patient.

These studies emphasize the importance of biochemical techniques in shedding light on the mechanism of hemolysis and the pattern of inheritance of congenital hemolytic disorders. Even though our knowledge of the etiologic role of many of these metabolic aberrations is incomplete, newer paths for exploration are becoming apparent. When these pathways are finally mapped, therapeutic application of this knowledge may become possible.

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APPROACHES TO THE CHEMICAL THERAPY OF TUMORS

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ALTHOUGH chemical agents capable of curing human cancer are not yet available, significant achievement in suppressive chemotherapy has been made. Moreover, some of the more dramatic clinical chemotherapeutic responses are seen in pediatric patients. It is the purpose of this review to examine recent advances in cancer chemotherapy, to provide a background for future work, and to explore conceptual approaches to the subject.

A number of general approaches has been employed in the development of agents for use in cancer chemotherapy. An active program in many laboratories involves the screening of large numbers of chemical compounds and materials of diverse origin in various model systems.[1] On the other hand, investigators[2] have studied tumors and nontumor tissues in the search for exploitable biochemical differences. This approach states that if a metabolic pathway could be found that was sufficiently unusual for tumor cells, it should then be possible to fashion an appropriate inhibitor of the pathway. However, the metabolism of the cancer cell has not been sufficiently elucidated to permit conclusions as to the most rational site of attack or the most effective type of molecule to prepare. Many investigators, preferring to avoid the empiricism of screening methods and impatient with a purely metabolic attack on the problem, have utilized what Hitchings[3] has termed "enlightened empiricism." In this way, available information regarding tumors, nontumor tissues, and growing material in general has been used as a basis for synthesis of compounds that would seem likely to have an antitumor action. The development of 6-mercaptopurine[4] represents a useful result of this approach to cancer chemotherapy. The concepts of sequential blocking[5] and concurrent blockade[4] which have been recently reported[6, 7] to result in improved effectiveness in the treatment of animal tumors are examples of the application of biochemical information and a theoretical approach to chemotherapy, although the empirical use of multiple agents in therapy antedates these concepts. Another general approach to the problem of developing new chemotherapeutic agents is through the study of the mechanisms of action of presently available compounds. Since there are agents that inhibit the growth of certain tumors in certain hosts, study of the pharmacology and chemical modification of these agents might be expected to lead to the development of superior agents.

The classes of drugs currently used in cancer chemotherapy include hormones, antibiotics, alkylating agents, and antimetabolites (Table I). The

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hormones have had an interesting place in the history of cancer chemotherapy. The demonstration by Huggins of the effectiveness of diethylstilbesterol in prostatic carcinoma was the first evidence that a synthetic compound of known chemical structure could influence the growth of a human neoplasm. Agents of this sort are effective in inducing regressions in tumors which are hormone dependent; thus, their effectiveness is limited to a few tissues of origin and to a few tumors of those tissues. The majority of tumors appear initially to be characterized more by their autonomy than their dependence and ultimate autonomy is in general. This area of chemotherapy has been reviewed by Huggins.[8] The use of *cortisone, related steroids, or corticotrophin* in the treatment of the acute leukemias of childhood would appear to be an example of another principle of hormone therapy in cancer, but the mechanism of suppression of leukemic cells or of normal lymphocytes by these steroids is unknown. In acute leukemia steroid therapy has been followed by prompt clinical improvement and objective hematologic remission in 30 to 50 per cent of children treated.[9, 10] The relatively shorter

TABLE I. AGENTS USED IN THE TREATMENT OF HUMAN NEOPLASTIC DISEASE

CLASS	TYPE OF AGENT	EXAMPLE	CONDITIONS IN WHICH USED
Antimetabolite	Folic acid antagonist	Amethopterin Aminopterin	Acute leukemias
	Purine analogue	6-mercaptopurine	Acute leukemias
	Pyrimidine analogue	5-fluorouracil 5-fluoro-2′-deoxyuridine	Carcinomata
Alkylating agents	Nitrogen mustards	Methyl-bis-(2-chloroethyl)amine(HN 2), tris-(2-chloroethyl) amine (HN 3)	Hodgkin's disease, lymphosarcoma bronchogenic carcinoma, polycythemia vera
		p-N-bis-(2-chloroethyl)-aminophenylbutyric acid (Chlorambucil, Leukeran)	HN 2-sensitive conditions, chronic lymphatic leukemia
	Ethylenimine derivatives	Triethylene melamine (TEM) Triethylene Thiophosphoramide (Thio-TEPA)	HN 2-sensitive conditions
	Alkyl sulfonates	1,4-dimethanesulfonyloxybutane (Myleran)	Chronic myelogenous leukemia
Nitrogen mustard analogues of metabolites	Amino acid derivatives	p-N-bis-(2-chloroethyl) aminophenylalanine	HN 2-sensitive conditions, seminoma
	Sugar derivatives	1,6-bis-(2-chloroethylamino)-1,6-dideoxymannitol (BCM)	HN 2-sensitive conditions
	Pyrimidine analogue	5-bis-(2-chloroethyl) aminouracil	HN 2-sensitive conditions
Hormones	Estrogens, androgens	Diethylstilbesterol	Endocrine-dependent tumors
	Adrenal steroids	Cortisone, prednisone	Acute leukemia
Antibiotics	Streptomyces products	Actinomycin D Mitomycin C	Some solid tumors

remissions associated with steroid therapy than with other chemotherapeutic agents have led to a major reliance on the antimetabolites in the treatment of acute leukemia. However, the rapid remissions associated with hormone therapy may be lifesaving, and a useful regimen involves the initiation of therapy in acutely ill children with a steroid hormone and an antimetabolite. In this way, clinical remission may be achieved during the interval before an antimetabolite could exert an antileukemic effect. Hormone therapy can be discontinued later. Bacterial toxins[11] were among the earliest agents said to be of use in the chemotherapy of cancer and the effectiveness of antibacterial antibiotics has served to focus attention on the study of *antibiotics* as inhibitors of tumor growth. Temporary regression has been observed in some human tumors following treatment with Actinomycin D,[12] and another antibiotic derived from streptomyces, Mitomycin C, has been reported to have induced tumor regression in patients treated in Japan.[13]

The agents most generally useful in the chemotherapy of cancer in experimental animals as well as in man are the alkylating agents and the antimetabolites. These compounds also have an intellectual appeal over the natural products and their derivatives, which is reflected in this review. On the other hand, research in all of these areas tends to provide cumulative information. The development of azaserine and 6-diazo-5-oxonorleucine represent the manner in which various areas of interest can be interrelated. Both compounds were found in the course of screening of crude filtrates of cultures of streptomyces and as such can be considered antibiotics or toxins.[14, 15] When these compounds were isolated and characterized,[16] they were both found to represent structural analogues of glutamine (Fig. 1). These agents were found to inhibit the growth of a number of tumors in experimental animals but they have not been useful in the treatment of human neoplastic disease. However, they have proved useful tools in the elucidation of pathways of metabolism.[17] Both compounds act as inhibitors of purine biosynthesis, de novo. In each case the inhibition is at the same step in the synthesis of inosinic acid in which α-N-formylglycinamidine ribotide is formed from α-N-formylglycinamide ribotide, glutamine, and ATP (Fig.

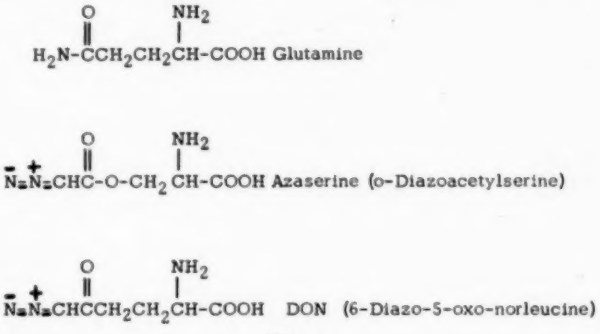


Fig. 1.

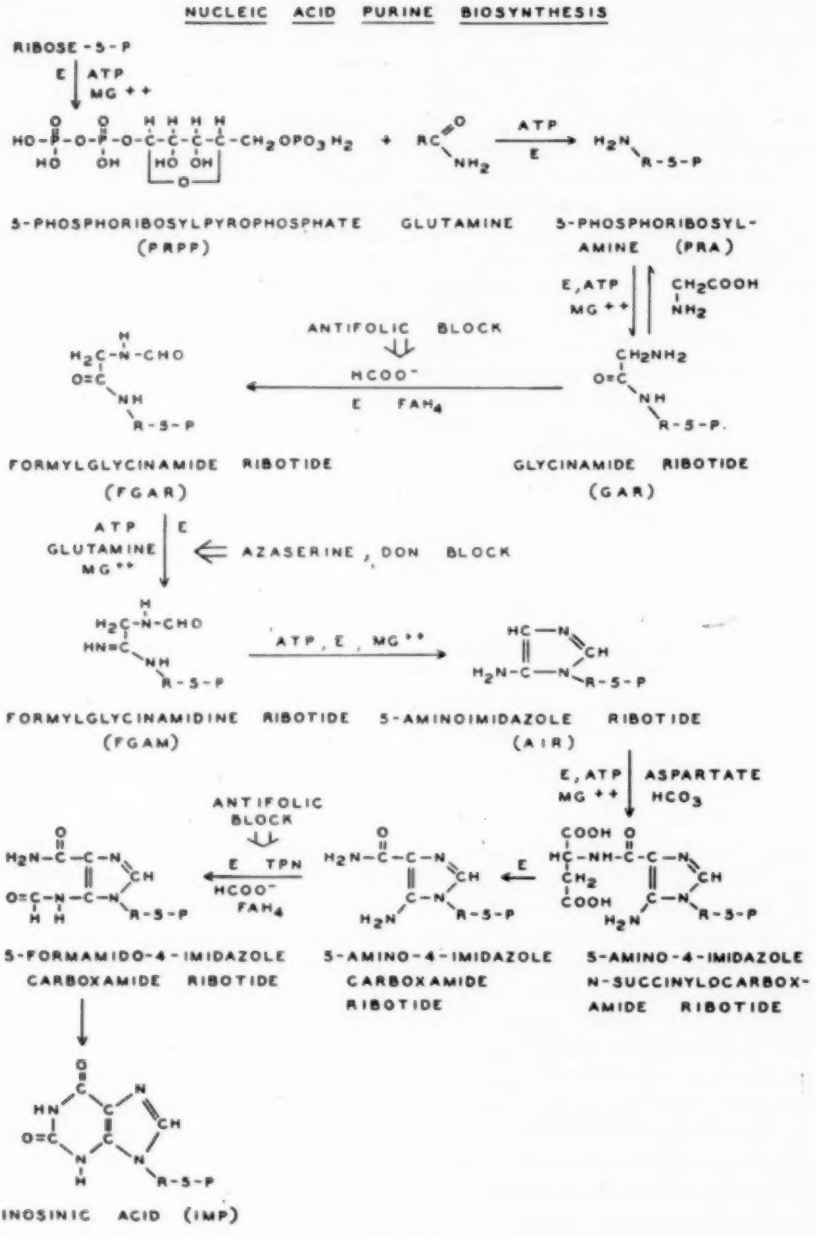

NUCLEIC ACID PURINE BIOSYNTHESIS
RIBOSE-5-P
E
ATP
MG++
5-PHOSPHORIBOSYLPYROPHOSPHATE (PRPP)
GLUTAMINE
ATP
E
5-PHOSPHORIBOSYL-AMINE (PRA)
E, ATP
MG++
CH2COOH
NH2
ANTIFOLIC BLOCK
HCOO-
E FAH4
FORMYLGLYCINAMIDE RIBOTIDE (FGAR)
GLYCINAMIDE RIBOTIDE (GAR)
ATP
GLUTAMINE
MG++
E
AZASERINE, DON BLOCK
ATP, E, MG++
FORMYLGLYCINAMIDINE RIBOTIDE (FGAM)
5-AMINOIMIDAZOLE RIBOTIDE (AIR)
E, ATP
MG++
ASPARTATE
HCO3
ANTIFOLIC BLOCK
E TPN
HCOO-
FAH4
5-FORMAMIDO-4-IMIDAZOLE CARBOXAMIDE RIBOTIDE
5-AMINO-4-IMIDAZOLE CARBOXAMIDE RIBOTIDE
5-AMINO-4-IMIDAZOLE N-SUCCINYLOCARBOXAMIDE RIBOTIDE
INOSINIC ACID (IMP)

Fig. 2.

2).[18] It was the study of systems so inhibited that resulted in the accumulation and identification of formyl glycinamide ribotide and glycinamide ribotide[19] as intermediates in purine biosynthesis. The elucidation of these pathways would appear to have relevance to cellular mechanisms of growth in general, and these inhibitors may in this way influence the development of chemotherapy.

mental constituents of the nucleic acids. Exogenous, preformed purines, and pyrimidines offer alternate substrates for the synthesis of the nucleotide precursors of the nucleic acids. The two formylation steps in the biosynthetic scheme are sites of inhibition by antimetabolites. Transfer of the single-carbon units at these sites requires the presence of a donor which is a derivative of tetrahydrofolic acid (Fig. 3),

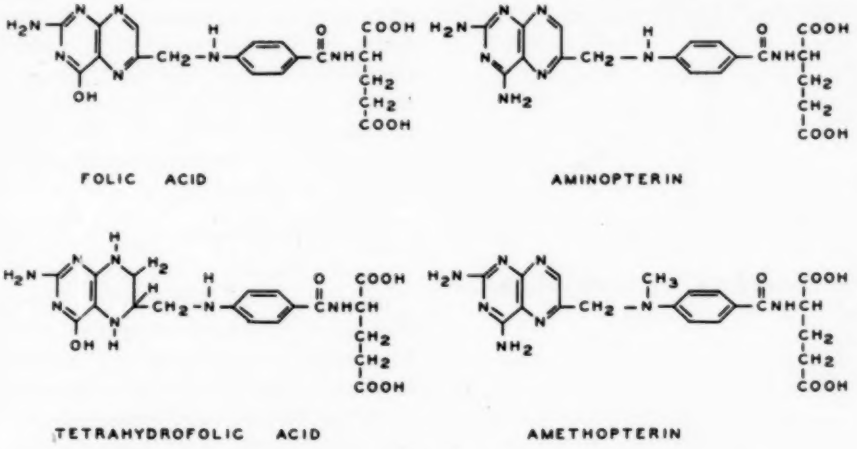


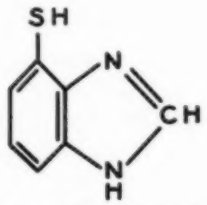
Fig. 3.

ANTIMETABOLITES

Folic-Acid Antagonists.—The development of the folic acid antagonists represented the first useful application of the antimetabolite principle to cancer chemotherapy. The action of these compounds and their effectiveness in acute leukemia have been the subjects of a number of reviews.[9, 10, 20] The antitumor effects of these compounds have been correlated with effects on the biosynthesis of the nucleic acid purines (Fig. 2). The inosinic acid formed in the scheme of de novo synthesis represented is in equilibrium with other purine nucleotides which are fundaprobably an active derivative of N-10-formyl- or hydroxymethylatetrahydrofolic acid.[21, 22] The folic acid antimetabolites (Fig. 3) contain an amino group instead of a hydroxyl group in the 4 position, with (amethopterin) or without (aminopterin) an N-10 methyl group. It has recently been found that folic acid is converted by two different enzymes first to dihydrofolic acid and then to tetrahydrofolic acid. It is the second enzyme that is inhibited by the antifolic compounds[23, 24] and of course formylation reactions are secondarily blocked.[25]

In children with acute leukemia clinical and hematologic evidence of

remission has been reported in 30 to 70 per cent of patients[9, 20] after 3 to 8 weeks of treatment with folic acid antagonists. Complete remission has been observed in 20 to 30 per cent of children treated. Amethopterin (Methotrexate) is the folic acid antagonist in most general current use.[9] It is administered by mouth in a dose of 2.5 mg. (1.25 to 5.0 mg.) per day. In the presence of remission maintenance therapy seems desirable.[10] However, treatment may be stopped, in which



6-MERCAPTOPURINE

Fig. 4.

case frequent examination of the bone marrow for signs of early relapse has been recommended.[9] As might be expected from the inhibition of so general a process as nucleic acid biosynthesis, a number of toxic reactions follow treatment with these compounds. The most common toxic manifestations include ulceration of the oral mucosa and depression of hematopoiesis which may be accompanied by the development of megaloblastosis. Diarrhea, melena, intestinal ulcerations, and alopecia are less commonly seen. The development of drug resistance ultimately limits the therapeutic effectiveness of these agents, suggesting the use by the tissue of alternate metabolic pathways. Skipper and co-workers[26] reported that while amethopterin inhibited the incorporation of C^{14}-labeled formate into the nucleic acids of susceptible leukemic cells, the drug produced an increase in the incorporation of the isotope of formate into the nucleic acids of the leukemic cells of a drug-dependent strain. Tivey[27] reported that the 50 per cent survival time in children with untreated leukemia was 4 months. This figure was doubled following the advent of the folic acid antagonists and the steroid hormones. The percentage of patients surviving longer than one year was 5 per cent prior to the development of these two forms of therapy and 24 per cent thereafter.[10] The folic acid antagonists have recently been reported by Rosenberg and associates[28] to induce remissions in certain cases of lymphosarcoma in childhood.

Purine Antagonists.—

6-Mercaptopurine: The synthesis of 6-mercaptopurine (Fig. 4) by Elion, Burgi, and Hitchings[4] represented part of a continuing study on the part of these investigators of purine metabolism and its inhibition as an approach to cancer chemotherapy. The mechanism of action of this compound is not known, but S^{35}-labeled 6-mercaptopurine was found to be incorporated into the nucleic acids of the viscera of mice[29] and of chronic myelocytic leukemic cells in man.[30] In mice the incorporation of formate into nucleic acids was inhibited by treatment with this compound, but the incorporation of adenine into nucleic acids was unaffected.[31]

The initial clinical trials were conducted by Burchenal and colleagues,[32] and since then considerable experience has indicated the effectiveness of the compound in producing remissions in acute leukemia. Clinical and hematologic remissions have been achieved

in approximately 50 per cent of pediatric patients treated with this agent alone.[9] The compound is administered by mouth, usually in a daily dose of 2.5 mg. per kilogram, and 3 to 10 weeks of therapy may be required before remission occurs. Toxicity, which limits the size of the dose that can be administered, is predominantly that of hematopoietic depression, although gastrointestinal lesions have been seen. Addition of this agent to those available for the treatment of acute leukemia increased the percentage of patients surviving longer than one year to 52 per cent.[10] The chemotherapeutic concept of sequential or multiple blockade by metabolic inhibitors at two or more sites in an area of cellular metabolism was originally proposed by Potter[5] and has received some recent support in the demonstration by Tarnowski and Stock[7] of an enhancement of carcinostatic effect on Sarcoma 180 in the mouse by using combinations of 6-mercaptopurine and azaserine. Preliminary clinical success with this combination of agents in the treatment of children with acute leukemia has been reported by Burchenal and associates.[9]

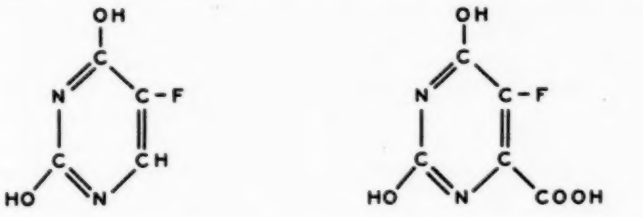


Fig. 5.

Fluorinated Pyrimidines.— The synthesis of 5-fluorouracil and 5-fluoroorotic acid (Fig. 5) by Heidelberger, Duschinsky, and Pleven[33] furnished the most recent addition to the list of antimetabolites of interest in cancer chemotherapy. The preparation of these compounds followed the studies of Heildelberger and co-workers[34] on the metabolism of uracil-2-C^{14} in the tumor-bearing rat. In vivo experiments indicated that uracil was utilized in the formation of nucleic acids to a greater extent in the Flexner-Jobling carcinoma and the intestine than in the liver. At the end of 24 hours after the injection of the tracer, 95 per cent of the isotope had been excreted, predominantly in the form of respiratory CO_2, indicating that the compound was actively metabolized. In vitro experiments demonstrated an extensive catabolism of uracil in the liver but very little catabolism in the tumor. These observations suggested the engaging hypothesis that a toxic analogue of uracil might be catabolized in nontumor tissues, allowing for a selective toxicity to the tumor which would be unable to destroy it. The hypothesis may be of general utility in chemotherapy, although of course one would be particularly interested in the catabolic activity of hematopoietic tissue and intestinal mucosa. Both compounds have been demonstrated to be effective in the treatment of tumors in a number of experimental animals. The

uracil derivative was generally more active than fluoroorotic acid. Clinical trials have been reported by Curreri and colleagues[35] and by Hall,[36] and striking regression has been observed in some solid tumors. Improvement noted has been temporary and the development of drug resistance has already been seen. The therapeutic index is apparently somewhat less than one; toxicity is predominantly to the bone marrow and the mucosa of the mouth and intestine. The sudden appearance of irreversible shock has been seen with a disturbing frequency and a number of therapeutic deaths have been recorded. On the other hand, the induction of significant regression of solid tumor masses in human patients is an important observation. Attempts are in progress to alter the biological properties of the compound by chemical modification. The desoxyribonucleoside of 5-fluorouracil appears to be more effective against certain animal tumors and less toxic than the parent compound.[37]

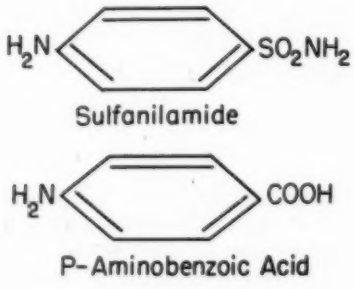


Fig. 6.

THE NATURE OF EFFECTIVE ANTIMETABOLITES

It is now clear that it is possible to fashion analogues of biologically important compounds which are capable of destroying solid tumors as well as leukemic cells in human patients. In the light of these observations it is of interest to review the development of the antimetabolite concept by Woods[38] and Fildes.[39] In the classical experiments of Woods[38] an active component was characterized from a bacterial extract capable of inhibiting the antibacterial action of sulfonamides, and p-aminobenzoic acid (Fig. 6) was found to compete effectively with the sulfonamides, preventing their effects on the bacteria. It was proposed that p-aminobenzoic was an important metabolite for the microorganisms and that the sulfonamide, an antimetabolite, prevented its utilization. The concept has stimulated the study of metabolism as an approach to pharmacology and a search for antimetabolites as therapeutic agents. Support for the original hypothesis came from the demonstration[40] that p-aminobenzoic acid made up a portion of the folic acid molecule (Fig. 3) and that sulfonamides prevented the synthesis of folic acid from p-aminobenzoic acid.[41, 42] Further, it was shown by Lampen and Jones[43] that bacteria which synthesized folic acid from p-aminobenzoic acid were sensitive to the action of sulfonamides, while bacteria which, like animals, require preformed folic acid were unaffected by the sulfonamides. It is evident from these data that the effectiveness of an antimetabolite depends not only upon a potent and specific ability to compete with a substrate for an enzyme but also on the presence of a distinct difference in metabolism or permeability between the target cell and host cells. The ideal antimetabolite for use in cancer chemotherapy might be a synthetic compound with structural similarity to a metabolic intermediate with a unique role in

the metabolism of tumor cells. Preferably, it should be a noncompetitive inhibitor of an enzyme system or a competitive inhibitor with a very high affinity for the enzyme.

ALKYLATING AGENTS

Nitrogen Mustards.— The other general class of compounds useful in cancer chemotherapy is that of the alkylating agents. The structures of sulfur mustard and the classical aliphatic nitrogen mustards are shown in Fig. 7. The original military code designations have been listed because simplicity has led to a persistence of these terms in general usage. Experience derived from World War I provided evidence that sulfur mustard was effective in the production of vesicant lesions in areas of contact,[44] and, early in World War II, it became evident that there was systemic toxicity as well.[45] The development of the tertiary amine mustards which were more readily absorbed through the intact skin served to focus attention on the systemic toxicity of these agents. The observation that injection of these agents was regularly followed by a profound toxic effect on lymphoid tissue led to the important experiments of Gilman, Goodman, and Dougherty.[46] These investigators demonstrated that in mice bearing transplanted lymphosarcoma and in patients with tumors of lymphoid tissue the injection of HN 2, methyl-bis-(2-chloroethyl)amine, or HN 3, tris-(2-chloroethyl)amine, was followed by dramatic regression of tumor masses and clinical remission. These observations constituted the first demonstration that it was possible for a synthetic compound to induce regression in human tumors arising from tissues not under endocrine control. The reported effectiveness of HN 2 and HN 3 was about equal, but it was the impression of the original investigators[47] that HN 3 was more difficult to work with from the point of view of

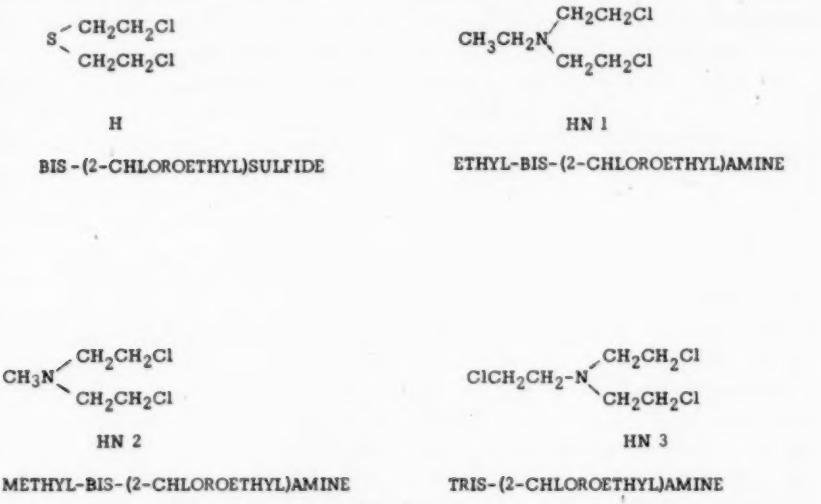


Fig. 7.

local reactions at the site of intravenous injection. A wide clinical and experimental experience has been accumulated with the methyl compound, HN 2, and it remains the standard against which new alkylating agents must be compared. It is administered intravenously in a dose of 0.4 mg. per kilogram, which may be divided into 2 or 4 successive daily doses.

The disadvantages of the compound have been consistently apparent. Only a few tumors or types of tumors are affected by the agent, and even among laboratory animals few cures of established tumors are recorded. On the other hand, even excluding vesicant effects, it is a very toxic compound.[48] In most animals the therapeutic index is 2 or less. Destruction of rapidly-dividing cells of bone marrow and intestine particularly limit the amounts of drug that can be administered. These considerations have led to a search for similar agents which might possess greater activity and lower toxicity or selectivity of penetration.

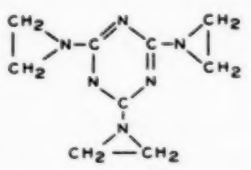
These compounds are highly reactive chemically and are capable of alkylating a number of biologically important groups, including amino, carboxyl, hydroxyl, sulfhydryl, imidazole, and phosphate groups.[49] Activity has been associated predominantly with compounds containing two or more active alkylating centers. This fact led to the cross-linking hypothesis[50] that these compounds could bind, as if with two hands, at two points in an intracellular system of parallel fibers. If sufficiently tight bonds were formed to produce interchromatid linkages, one might imagine that changes occurring in mitosis could account for the production of the fragmented and "sticky" chromosomes observed cytologically by Koller and co-workers[51, 52] and Friedenwald and Buschke[53] following nitrogen mustard treatment. The cross-linking hypothesis has been seriously questioned as a result of the demonstration that certain monofunctional compounds have similar biolgical effects[54] and the experiments of Alexander and colleagues[55] in which HN 2 and sulfur mustard had no cross-linking activity for wool fibers. However, the concept may still have relevance to the mechanism of intracellular action, and it has been valuable in that it has led to the development of other biologically active agents.

Other Alkylating Agents.—Search for chemotherapeutic agents among derivatives of ethylenimine was suggested by the fact that the initial reaction of the aliphatic nitrogen mustards in water is the loss of chloride with the formation of a highly reactive cyclic ethylenimonium ion.[56] Among the first of these compounds to be employed in cancer chemotherapy was triethylene melamine (TEM)[57, 58] (Fig. 8) which has been used in the wool industry as a cross-linking agent. The triethylenimino-phosphoramides, both the oxygen and the thio analogue (Fig. 8), have been more recently developed.[59, 60] A number of difunctional alkyl sulfonates have been prepared by Haddow and Timmis,[61] among which 1,4-dimethanesulfonyloxybutane (Myleran) has been shown to have clinical utility. A large series of aromatic nitrogen mustards has been synthesized by Ross and co-workers[49] and studied by Haddow and co-workers in experimental animals[62] and in man.[63] The ethylenimines, the alkyl sulfonates, and the aromatic mustards possess an advantage over the aliphatic mustards

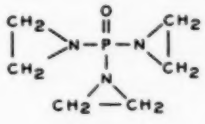
in that they can be administered by mouth. However, none of these agents has been shown to have any other clear advantages over HN 2 in the treatment of tumors of lymphoid tissue, and the original compound has not been replaced in general clinical use. On the other hand, two instances of specificity of action have emerged from these studies. Myleran has been found to be particularly toxic to granulocytes[61] and has been used effectively in the treatment of chronic granulocytic leukemia. The nitrogen mustard derivative of p-aminophenylbutyric acid (Chlorambucil, Leukeran)[64] is more effective against circulating lymphocytes[65] and is said to be particularly effective in chronic lymphatic leukemia.[66] This evidence of alteration in cytotoxic specificity associated with structural modification has served to stimulate further investigation of chemical alteration and structure-activity relationships among the alkylating agents.

NITROGEN MUSTARD DERIVATIVES OF BIOLOGICALLY ACTIVE COMPOUNDS

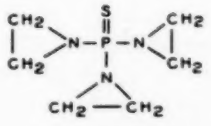
Useful agents for the chemotherapy of cancer have resulted from the preparation of antimetabolites and alkylating agents. One of the more recent developments in this area has been the synthesis of compounds designed to be at the same time alkylating agents and structural analogues of important intermediates of cellular metabolism. In these compounds the intermediate might serve as a carrier for the cytotoxic moiety, providing some selectivity of entry into certain areas of certain cells. The work of a number of investigators has indicated that amino acids are actively utilized by tumor cells in vitro[67-70] and in vivo[71-75]; the recent experiments of Busch, Davis, and Anderson[76] have shown that while nontumor tissues selectively incorporate the isotope of C^{14}-labeled lysine into cytoplasmic proteins, tumors carry out selective labeling of nuclear proteins. These observations suggest that



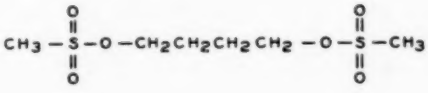
TRIETHYLENE MELAMINE
TEM



TRIETHYLENE PHOSPHORAMIDE
TEPA



TRIETHYLENE
THIOPHOSPHORAMIDE
Thio TEPA



1,4 - DIMETHANE - SULFONYLOXYBUTANE
MYLERAN

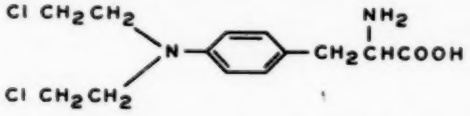
Fig. 8.

amino acids might be appropriate carrier structures for cytotoxic molecules. The findings that transplantable tumors of the rat, unlike nontumor tissues, are capable of incorporating the isotope of previously labeled plasma proteins into cytoplasmic particulates in vivo[71, 77] suggest the possibility that greater specificity of antitumor action might be achieved by the preparation of cytotoxic derivatives of molecules such as peptides or proteins.

The synthesis by Bergel and Stock[78] of p-N-bis-(2-chloroethyl) aminophenylalanine (Fig. 9) represented the first example of the deliberate addition of a cytotoxic alkylating group to an amino acid residue. These investigators have reported the synthesis of the DL-amino acid, resolution into the D- and L- isomers, and synthesis of the DL-C^{14}-labeled compound.[79] Administration of the compound to experimental animals has been followed by inhibition of the growth of a number of experimental tumors,[52] including some tumors that are not responsive to the classical nitrogen mustards.[80] Administration of the phenylalanine mustard to mice bearing therapeutically resistant Harding-Passey melanoma or the Cloudman melanoma was followed by a striking therapeutic effect.[81, 82] Activity against these tumors suggests that the compound may function as an analogue of tyrosine or dihydroxyphenylalanine. In these studies as well as those of Koller and Veronesi[52] the L- form was considerably more effective than the D- isomer, and the nitrogen mustard derivative of p-aminophenylbutyric acid was inactive. These observations suggest that penetration of the mustard to the site of action or the attack on the tumor involves the participation of proteins with affinity for the alpha amino group in the L-configuration.

Toxicity similar to that produced by HN 2 has been observed, but the therapeutic index of the phenylalanine derivative is five times greater. Galton and associates[83] have reported conservative clinical results, generally similar to those found with the phenylbutyric acid mustard in patients with Hodgkin's disease and lymphosarcoma. On the other hand, Larionov[84] has reported his experience of dramatic clinical remissions, particularly in patients with seminoma, and a broader antitumor spectrum than that of the other mustards. In extension of these investigations Bergel and Stock[85] have prepared some α-N-benzoyl-dipeptides of phenylalanine carrying a mustard group on the phenylalanine moiety. While these compounds were inactive against the Walker 256 carcinosarcoma, two synthetic α-N-formyl dipeptides of phenylalanine with mustard groupings in the para-position were reported by Larionov and Sofina[86] to be as effective as the phenylalanine mustard in a number of rodent tumors. Izumi[87] has synthesized the N-bis-(2-chloroethyl) derivatives of glycine, alanine, and taurine, among which the alanine mustard (Fig. 10) was the most effective compound. These compounds were all less toxic than HN 2 and had no vesicant action for human skin. Assayed against the Yoshida ascites sarcoma, a therapeutic index was reported for the alanine mustard that was 30 times that of HN 2. In rats bearing the Walker tumor this index was greater than that of HN 2 but less than that observed using

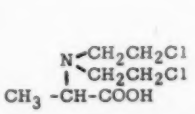
the phenylalanine mustard. An agent which structurally resembles the phenylalanine mustard is γ-[p-NN-bis-(2-chloroethyl) aminophenyl]-α-aminobutyric acid (Aminochlorambucil) which was synthesized by Davis, Roberts, and Ross.[88] This agent has been found to produce a 5 to 10 times greater fall in circulating human lymphocytes[89, 90] with less bone marrow damage than does chlorambucil, suggesting that it might be particularly effective in the treatment of chronic lymphocytic leukemia. In a preliminary report clinical benefit has been noted in the treatment of patients with Hodgkin's disease and reticulosarcoma.[90] More recently, Bergel and Wade[91] have prepared a nitrogen mustard derivative of serine, o- [-N-bis-(2-chloroethyl) carbamoyl] -DL-serine, a compound of different general structure from other amino acid mustards (Fig. 10). It is relatively nontoxic and the therapeutic index appears to be at least 10 as tested in rats bearing the Walker tumor.



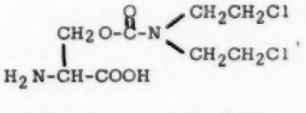
p-N-BIS-(2-CHLOROETHYL) AMINOPHENYLALANINE

Fig. 9.

The synthesis of a series of ethylenimino and 2-chloroethylamino derivatives of sugars has recently been reported from Hungary by Vargha and co-workers.[92, 93] Among these compounds, the N-bis-(2-chloroethyl) derivative of glucosamine[92] was found to produce inhibition of the Guerin tumor but in doses which killed the animals. The most active of these compounds was 1,6-di-(2-chloroethylamino)-1,6-dideoxymannitol (BCM)[93] (Fig. 11). This compound is of interest as a novel alkylating agent because it contains two secondary nitrogen

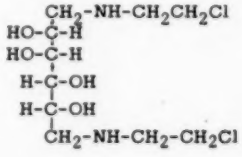


N-BIS-(2-CHLOROETHYL) ALANINE



O- [N-BIS-(2-CHLOROETHYL)CARBAMOYL] -SERINE

Fig. 10.

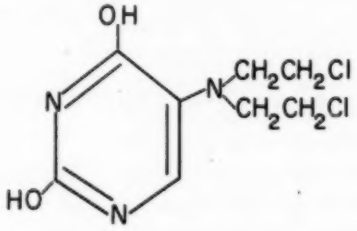


1,6-DI-(2-CHLOROETHYLAMINO)-1,6-DIDEOXYMANNITOL(BCM)

Fig. 11.

atoms and it is also a mannitol analogue. Inhibition of tumor growth was observed in a number of animal tumors. The effects of this compound were felt to represent superiority over HN 2 in a smaller toxicity as well as a broader therapeutic effectiveness.[94]

The most recent addition to this group of compounds is 5-bis-(2-chloroethyl)aminouracil, a pyrimidine derivative synthesized by Lyttle and Petering[99] (Fig. 12). The active incorporation of uracil into nucleic acids and the deficient catabolic activity to



5-Bis-(2-Chloroethyl) Aminouracil

Fig. 12.

Clinical effectiveness[95, 96] has been reported in patients with Hodgkin's disease, chronic lymphatic leukemia, and lymphosarcoma. Pretreatment of animals with this compound prevented the development of metastatic implants following the intravenous injection of tumor cells.[95] These experiments suggest the use of the agent in surgical patients to prevent the spread of metastases at operation, as has recently been reported by Economou, Mrazek, and Cole[97] using HN 2.

The synthesis by Hirschberg and colleagues[98] of 2-[di-(2-chloroethyl) aminomethyl]benzimidazole represents the addition of a mustard group to a compound which is itself an analogue. The resultant molecule might be considered a possible purine antagonist. It has been found to inhibit the growth of four different tumors of mice, two of which were unaffected by HN 2 or p-N-bis-(2-chloroethyl)aminophenylbutyric acid.

uracil noted in tumors[34] would appear to favor this compound in a rational approach to chemotherapy. The compound has been shown to be effective in the treatment of a number of experimental tumors, including some resistant to the classical chemotherapeutic agents. In rats bearing the Walker tumor therapeutic effectiveness has been observed in doses of one seventh the LD_{50}.[100, 101] Clinical trials are in progress and good responses have been observed in patients with Hodgkin's disease and with chronic leukemias.[102, 103]

SUMMARY

Drugs have been developed which are capable of inhibiting the growth and in some instances curing experimental tumors in animals, and some of these agents are useful in the palliative suppression of human neoplastic disease. Among these agents

are hormones, antibiotics, antimetabolites, and alkylating agent. Discussion has been centered around the latter two classes of compounds, for in these areas the application of a conceptual approach to the preparation of new agents is more readily apparent. In a number of recent instances information derived from investigation of cellular metabolism has been applied to the synthesis of new compounds with antitumor effects. Although chemotherapeutic inroads into the problem of human neoplastic disease have as yet been small, these observations should serve as a stimulus for further definition of the metabolism of neoplastic cells and of the mechanisms by which available agents exert their cytotoxic effects.

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THE OCCURRENCE OF ECTODERMAL DYSPLASIA AND CORNEAL DYSPLASIA IN ONE FAMILY

An Inquiry Into the Mode of Inheritance

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FAMILIAL ectodermal dysplasia is an uncommon disorder which is characterized classically by the triad of anhidrosis, hypotrichosis, and dental hypolasia. The affected individuals may be separated into three overlapping categories which reflect the severity of their affliction: (1) asymptomatic persons; (2) minimal to moderate stigmata; and (3) those manifesting the complete syndrome. In the complete syndrome which is seen far more commonly in males there is almost total absence of sweating, with consequent difficulty in body temperature regulation, scant hair to alopecia totalis, and a few abnormally formed teeth to complete adontia. There is frequently some degree of albinism, a smooth, glossy skin with wrinkling in the areas of loose skin, eczematid dermatitis, thickened lips with a poorly defined vermilion border, atrophic nasal and pharyngeal mucous membranes, ungual defects, absent mammae, saddle nose, frontal bossing, and mental retardation. Histologic examination of the skin in those individuals with the complete syndrome consistently reveals absence of sweat glands, often absence of hair follicles, and frequently absent or rudimentary sebaceous glands. Individuals in the second category show varying degrees of reduction of hair, abnormal teeth, and some recognize anhidrotic areas on their bodies.[2, 3]

It will be noted from the above description of symptoms that although the disease is called ectodermal dysplasia there are in fact also derivatives of both mesoderm and endoderm which manifest involvement. However, all defects could probably be explained on the basis of an abnormality in the metabolism of ectoderm, with the resulting secondary alteration in the nonectodermal elements.

On the basis of the clinical and symptomatic differences which are quite dramatic, Weech[1] proposed the descriptive terms hidrotic and anhidrotic forms but suggested they were variant manifestations of the same disorder. In support of this thesis one encounters reports of individuals with the anhidrotic form who, either through their own observation or by testing the capacity of their entire skin surface to sweat, were able to sweat in certain localized areas.[2-5] In a very careful study of a patient who symptomatically was of the anhidrotic type Felsher[6] showed the patient to have few areas of the skin where some sweating occurred, however, at a rate approximately 5 per cent of the

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normal. More convincing is the occurrence of relatives of a patient with the anhidrotic form who have the hidrotic form of this condition. Wagner[5] reported a mother and a daughter who were ostensibly anhidrotic but they were shown to sweat in a few delimited areas, as demonstrated by the Minor starch iodine test and skin resistance studies.

Many authors have been interested in the mode of inheritance of this disorder. The striking feature has been the predominance of males who have the anhidrotic form of the disease and the fact that many of the mothers of these patients are apparently normal, although the disease could be shown to occur in the maternal lineage.[4, 7, 8] It was therefore suggested that the condition was sex-linked, the female being a carrier.[3, 4, 8] However, there is a report of the disease being transmitted through the father to his sons.[9]

We have investigated several families having members with ectodermal dysplasia, correlating the clinical manifestations with the pattern of sweating over the entire body surface as determined by the skin resistance pattern. This report deals primarily with one of these families in which both ectodermal dysplasia and a familial corneal dystrophy occur. These studies clarify the mode of transmission of ectodermal dysplasia in this family.

Using an abnormal skin resistance pattern as the frame of reference, there emerges a clinical spectrum from the apparently normal individual who has a few small areas on the skin which do not sweat to the full syndrome where there is no significant sweating. Abnormalities of the hair and teeth also show variable involvement, which in the experience here is not necessarily correlated with the degree of involvement reflected by the skin resistance pattern.

The corneal dystrophy which occurs in this family is the parenchymatous form of the granular type (Groenouw type 1[10]). This type usually begins in the first decade and the opacities usually become readily visible by the twentieth year. The pupillary region becomes more or less covered by whitish opacities of variable shapes. The dystrophy involves only the central portion of the cornea. The peripheral and deep central layers remain untouched. From the third to the fourth decade the opacities cover about ⅔ of the central area, but the decrease in vision is not as great as in the other classical forms, and often the patient is able to continue working to an advanced age.[11] Bücklers[12] classified the corneal dystrophies and indicated that the granular and lattice-like types were transmitted as a dominant and the macular type as a recessive trait.

In the family under investigation two pathologic mutant genes exist, both give evidence of being transmitted as dominant traits.

METHODS

The skin resistance was measured by a Whelan and Richter dermometer.[13] This device employs a constant, low-voltage galvanic current and the resistance found is a function of polarization and counter currents generated in the tissues. This resistance has been shown to be concentrated in the skin, controlled in part by the nervous system, mediated by the sympathetic component, and to vary inversely with sweat gland activity.[14, 15] Areas of hypohidrosis or anhidrosis are indicated by marked increase in

resistance. The anhidrotic areas on the first two patients (44 and 45, Fig. 1) were confirmed with the Minor starch iodine method.[16]

individuals have been tested in the psychobiology laboratory. No localized areas of high resistance (non-sweating) have been found on the

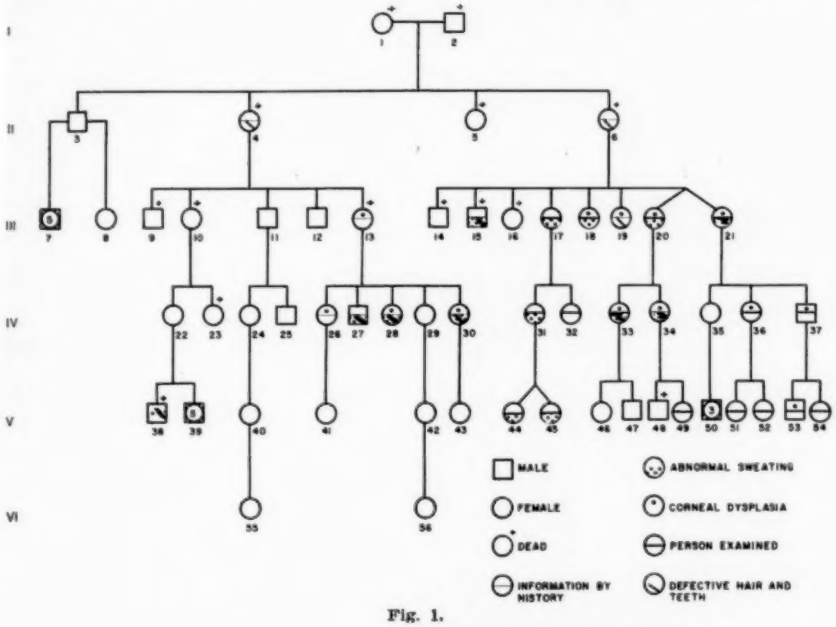


Fig. 1.

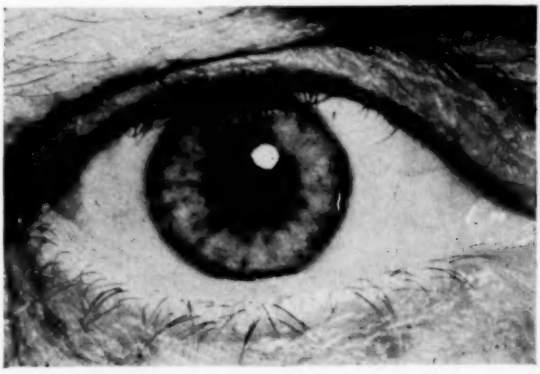


Fig. 2.

Fig. 1.—The lineage of the reported family is illustrated in this diagram.

Fig. 2.—The eye of patient 28 in which the corneal lesions are clearly visible. These are demonstrative of the type of lesion seen in this family. (The photograph is through the courtesy of Dr. Louis Girard.)

Over a period of years the skin resistance patterns of several thousand skin of any individuals without a specific nerve injury.

The corneal dystrophy of individuals 18, 19, 20 and 21 (Fig. 1) has been repeatedly examined by Dr. John Billingsly, Kansas City, Kansas. An ophthalmologic examination was performed on M. G. (28) by Dr. Louis Girard, Baylor University, who observed the corneal lesion (Fig. 2). Both of these ophthalmologists concur in the diagnosis of Groenouw's dystrophy. Dr. Edward Maumanee, Johns Hopkins Hospital, examined patients 31, 44, and 45 and found no evidence of corneal dystrophy.

CASE REPORTS

S. J., 45 (Fig. 1), was an 8-year-old white girl twin who was brought to the dispensary because of poor teeth. The history obtained indicated that she has had a full complement of deciduous teeth of normal appearance and that only three permanent teeth had erupted. She was the first born of monozygotic twins, and she has always been smaller than her sister. Her hair has always been thinner than normal and brittle. She was able to perspire but found that she became exhausted easily during play in hot weather.

The pertinent physical findings consisted of thin hair which was dry and brittle. The eyebrows showed thinning. There was some wrinkling of the skin under the eyes and irregular thickening of the skin of the nose. The lips were thick and the vermilion border was not distinct (Fig. 3). There was only one tooth, the other two having been extracted (Fig. 4). The skin over the body was smooth and soft but no gross evidence of abnormality was found. There was no abnormality of the nails or eyes.

Studies.—The hemogram, urinalysis, serologic tests for syphilis, and tuberculin test were all normal. The skin-resistance pattern revealed irregular areas of increased electrical resistance (Fig. 5). These areas of anhidrosis were corroborated by the Minor starch iodine test. Dental x-rays showed a single molar unerupted. To test the function of other ectodermal elements, an EEG, audiogram, and an I.Q. test were performed. The former were normal; the I.Q. was 116. The blood grouping studies revealed: A_1, CDe/c-e, M^+, N (–), Kell, and $Duffy^+$.

N. J., 44, was the twin sister of the above-mentioned patient. She accompanied her sister to the dispensary but had no complaints. She had no difficulty in hot weather. Like her sister her deciduous teeth were said to have been normal; however, not all deciduous teeth lost had been replaced by permanent teeth and several of the permanent teeth present were abnormal.

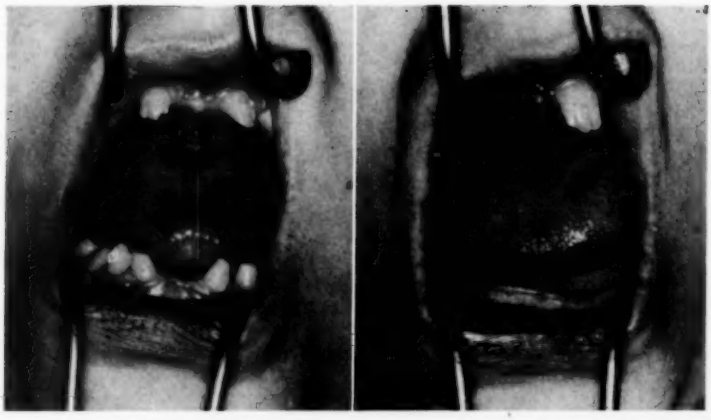
It was apparent on inspection that the twin's hair was also thin and brittle, although less markedly so than her sister's. Wrinkling of the skin beneath the eyes was present and there was the irregular thickening of the skin of the nose (Fig. 3). The eyebrows were thinned. The abnormality of the teeth is shown in Fig. 4. The lips were normal in appearance. Her skin was also smooth and soft without apparent abnormality. There was no abnormality of the nails or cornea. Fig. 6 illustrates the difference in stature and body build of the two girls.

The studies performed were the same as for her sister. The results were the same except for the I.Q. test, which was 119, and the extent and pattern of the islands of anhidrosis (Fig. 5). Specifically, it should be stated that the blood groups tested were identical.

E. J., 31, is the 34-year-old mother of the above patients. She had no symptoms or abnormal findings except ungual moniliasis, proved on culture. Her skin resistance pattern is illustrated in Fig. 5. A complete ophthalmologic examination revealed no abnormality.

Left *Right*

Fig. 3.—To the left is N. J., 44. Note the fine hair, diminished eyebrows and lashes, wrinkling of the skin under the eyes, and the less apparent roughening of the skin of the nose. The vermilion border of the lips is fairly well delineated. On the right is her twin, 45, who shows all of the aforementioned findings but greater in degree. In addition, she also shows somewhat thickened lips with less definition of the vermilion border.



Left *Right*

Fig. 4.—The teeth of patient 44 are on the left. There are a number of teeth which failed to erupt and most of those present are abnormal in shape. On the right her twin, 45, shows a single tooth present and this one is abnormal in shape.

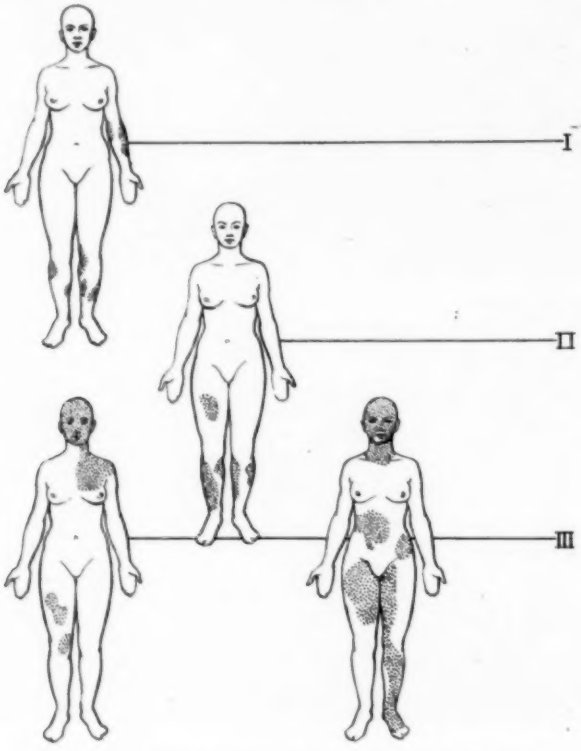


Fig. 5.—A schematic diagram of the areas of anhidrosis as determined by skin resistance on patients 17 (I), 31 (II), and 44 and 45 (III). Patient 44 is to the left, 45 is to the right. The patients numbered 17 and 31 were completely asymptomatic and unaware of the small anhidrotic areas.

M. B., 17, is the 64-year-old maternal grandmother of the twins. She had no symptoms relative to ectodermal dysplasia or Groenouw's dystrophy. She has had diabetes for 10 years, teeth, hair, skin, and nails. The cornea had small whitish specks on the central portion. Skin resistance studies showed small, localized areas of anhidrosis (Fig. 7).

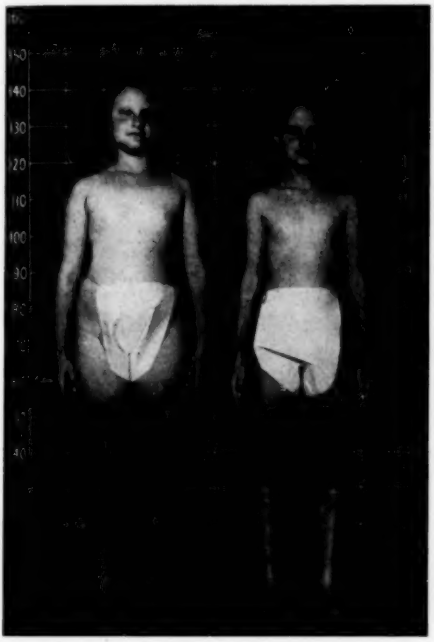


Fig. 6.

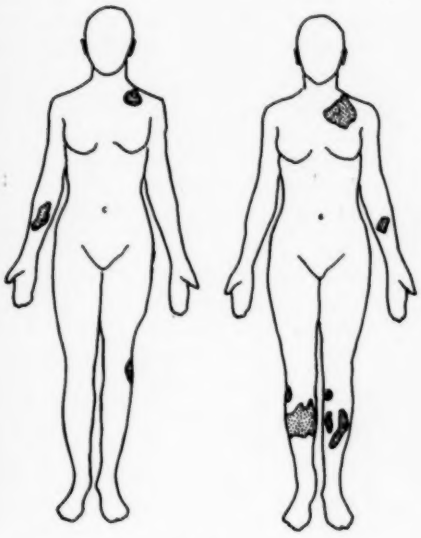


Fig. 7.

Fig. 6.—Patients 44 and 45 standing against a metric grid. The twin on the left shows more advanced growth and development than the twin on the right. The twin on the right shows more extensive manifestations of ectodermal dysplasia as judged both by clinical criteria and the skin area showing anhidrosis.

Fig. 7.—A schematic representation of the sweating pattern of patients 20 and 21, apparently identical twins. Note the dissimilarity of degree of involvement and the lack of "mirror image" correlation. Patient 21, on the right, has clinical evidence of ectodermal dysplasia, namely, thin hair and abnormal teeth, whereas the twin does not have any detectable stigmata other than the small islands of anhidrosis.

controlled by diet. Ophthalmologic examination was negative; her skin resistance pattern is illustrated in Fig. 5.

C. C., 20, was a 67-year-old white woman who was a great aunt to the above patients. She too was one of twins. She was examined in the course of the family study. She had a 10 year history of diabetes. She had noticed spots on her cornea, progressing over the previous 10 years. She had no history of heat intolerance. Physical examination revealed normal

M. C., 21, a 67-year-old white woman, was the twin of the above patient. She had abnormal permanent teeth and 3 which did not erupt prior to having them all extracted. She had noticed that her hair had always been thinner than that of her twin. She was aware of spots on her cornea, progressing over the last 20 years. Both 20 and 21 had the blood type A_1, c & e/c&e, M^+, N^+, Kell, and Duffy (–). On examination her hair was thin and brittle. She had full

dentures. Her skin was smooth. The irregular whitish spots over the central portion of her cornea were somewhat more extensive than those of her sister.

Patients 15, 27, and 38 represent the 3 male members of the family known to be affected with ectodermal dysplasia. Patients 15 and 38 died under 2 years of age with infection and hyperpyrexia. Both had scant hair, poor teeth, lack of sweating, and marked intolerance to heat. A photograph of 15 revealed the typical facies of a boy with ectodermal dysplasia. Patient 27 is a 54-year-old man with scant, fine hair, very poor teeth which were removed to permit full dentures, and marked intolerance to heat. Indeed, he had found by experience that he had to spend the day in the bathtub during the hot days of the summer. In the fall, when it became cooler, he helped with the harvest.

The rather marked difference in expressivity of the gene in apparently identical twins (20 and 21; 44 and 45) is worthy of emphasis. It can be seen from Figs. 5 and 7 that there is not mirror-image relation of the anhidrotic areas from one twin to the other. Furthermore, the clinical findings such as the degree of involvement of the hair and teeth is quite dissimilar. There is a correlation in patients 44 and 45 between degree of involvement and growth. That is, the twin showing the greater involvement as reflected by the size of the areas of anhidrosis and the degree of abnormality of the hair and teeth is shorter and less well developed than her less involved twin. This may be coincidence or cause and affect.

It can be seen that the ectodermal dysplasia and the corneal dystrophy occur concomitantly but they also occur independently in 2 to 3 generations. There are insufficient data to determine whether there is linkage with crossing over or nonlinkage.

RESULTS

The incidence of evidence of ectodermal dysplasia and corneal dystrophy in the family is shown in Fig. 1. Clearly, neither condition is inherited as a recessive gene. As can be seen from Fig. 1, the frequency of involvement in each generation is approximately the same even in these very small numbers. Only a dominant characteristic could give this incidence. It is apparent that the seeming incidence of individuals affected with ectodermal dysplasia is quite grossly altered by being able to discern those asymptomatic individuals exhibiting only small areas of unnoticed anhidrosis, i.e., discerning those with limited expressivity of gene effect. All of the individuals in this family showing limited expressivity are females. We have not encountered a male with limited expression to the extent commonly found in the female. Such examples in males are reported, however.[2, 9, 18] It is curious that this family should have such a predominance of females (38:14). An adequate history relating to miscarriages has not been obtained. It should be noted too that our data do not rule out sex-linkage of ectodermal dysplasia; that is to say we have no examples of the trait being passed from father to son. The possibility that this sex-linked hypothesis may be incorrect rests on the family cited by Brain,[9] where an affected father transmitted the disease to two sons. It would be of great value to have other incidences of father-to-son transmission reported wherein the

studies utilized currently available objective methods of determining sweating capacity.

The transmission of the corneal dystrophy is more clearly delineated. Fig. 1 shows approximately equal frequency of corneal dystrophy in generations III and IV. Such a distribution is compatible only with a dominant trait. The transmission of the dystrophy from the father, patient 37, to the son, patient 53 (Fig. 1), rules out sex-linkage for the gene causing corneal dystrophy.

There is clear evidence of sorting out or segregation of these two genes within this family. Thus, patient 21 had both conditions, but a son and a daughter have only the corneal dystrophy. Patient 17, a sister of 21, had no corneal dystrophy but did show evidence of ectodermal dysplasia. Those of her children and grandchildren affected had only ectodermal dysplasia, none had corneal dysplasia. Tabulating those members of the family in whom there is sufficient data, 9 have evidence of having both mutant genes, 10 only that of ectodermal dysplasia, and 4 only eye involvement.

DISCUSSION

The data from this family suggest that ectodermal dysplasia is transmitted as a dominant trait when mapping of the sweating capacity of the total surface area is used as the indicator of involvement. If generalized hypohidrosis, defective hair and teeth are the criteria used, as heretofore, a very different pattern of transmission might be concluded.[17]

In a recent publication[18] the use of a palm print on the silver nitrate plate was recommended for the diagnosis of anhidrosis. Although this may be satisfactory for those patients more severely affected, it is likely to miss the more frequent partially affected individuals. In the members of our kindred the 3 male members had extensive involvement. Among the females a great deal of variation is evidenced. In the members of the family shown in Fig. 5 the grandmother and mother were asymptomatic, showing only small discrete anhidrotic areas. The twin sisters (44 and 45) showed more extensive involvement, but, despite the fact they apparently were identical twins, showed discrepant degrees of involvement between themselves. This discrepancy was also seen in the twins 20 and 21 (Fig. 7) who were identical according to blood grouping. No affected female in this family showed the degree of involvement of any of the 3 involved males.

The suggestion that the gene causing the condition is sex-linked looks very appealing from the data in the literature. The most frequent combination reported is that in which an apparently normal mother has a son with the anhidrotic form of the disease. There are, also, quite frequent references of mother-to-daughter[5] or of father-to-daughter transmission.[1, 4] In contrast, Brain[9] has reported an affected father having two affected sons, which would rule out sex-linkage. One must consider the possibility that Brain's family represents a different mode of transmission from that usually seen in families with ectodermal dysplasia, which could signify that a different gene is involved.

In attempting to classify the mode of transmission of ectodermal dysplasia one immediately encounters the problem of defining dominance for any particular trait in question. A

gene is said to be dominant when a single allele effects full expression. This can be only a surmise in human genetics, for it is extremely rare that anyone has had the opportunity to see and study examples of individuals who possess two mutant alleles, either of which in single dose would cause disease. It is apparent that in ectodermal dysplasia there may be full expression in the presence of one mutant gene but there is also great variation in expression of this gene. The term "incomplete dominance" would be applicable.

As judged from our own experience and those cases reported, there can be little question that there is a very real proclivity of the male toward more complete expression of the gene. Thus, the sex of the individual in some way affects the expression of the gene. It is doubtful that hormones play a role in this difference in expression of the abnormal gene since there is no manifestation of disordered sexual development or function. Genetic rather than external influences would appear to be more likely since an alteration resulting in the failure of the development of hair, sweat glands, and sebaceous glands would have to be operative sometime prior to the second month of gestation, that is, prior to the time of the development of the anlage of the sweat glands, hair follicles, sebaceous glands, and teeth. If this were a sex-linked gene, one could readily visualize that the affected male would have an X chromosome with the abnormal gene and that there would be no normal allele in the Y chromosome to oppose or modify the untoward effect. In the female one X chromosome would contain the abnormal ectodermal dysplasia allele but the second X chromosome would be normal, containing the normal allele, and, hence, modify the effect of the mutant gene. If, on the other hand, the disease is sex-influenced, which would be the case if Brain's report is accepted, then the explanation of the modus operandi is more difficult. The simplest and most direct surmise would be that of a modifying gene or genes located in the X chromosome. This modifying gene would have the effect of suppressing the expression of the ectodermal dysplasia gene which is located in an autosomal chromosome. Logically the presence of 2 X chromosomes, as found in a female, with two modifying genes would give greater suppression than could be attained in the male where only one X chromosome exists. This is suggested from the observation that females may show relatively marked involvement (expression of the mutant gene) and that the X chromosomes vary with respect to the suppressor genes.

The next point that is puzzling when considering the genetics of any anatomic defect is how the "one-gene-one-enzyme" concept can possibly be applicable. In ectodermal dysplasia there are a number of ectodermal elements and even some mesodermal elements which are abnormal. How can such diverse alterations as absence of sweat glands, absent to rudimentary sebaceous glands, total absence to a few abnormally shaped teeth, alopecia to thinning of hair, frontal bossing, saddle nose, and decreased pigmentation be unified under an alteration of a single biochemical reaction? At this point one must recall that the organism is the product of many thousands of genes acting synchronously. The phenotype reflects the blending of

synchronized synthetic processes resulting from all of the genes in the genome. All genes interact with one another through their products and, hence, are modifiers of the activities of one another. Embryonic development is a sum result which must ultimately be accounted for on the biochemical level. Thus, for the normal development of a sweat gland, several hundred biochemical reactions may be involved. It is not only essential that the gene responsible for each enzyme be present but also that each reaction or product be present at the proper time. The whole series of reactions must be unerringly synchronized. Absence of a gene or a series of genes involved in one or more steps of this organized process or imperfect synchronization in the earliest phase of ontogeny of the particular tissue or gland could result in total absence or varying degrees of maldevelopment, depending on the temporal relation of the activity in question to ontogeny or the extent of reduction of function of the enzyme involved. Hypothetically one could visualize that the elaboration of compound *c* would be necessary for step C in the normal biochemical sequence of the development of ectodermal elements and that without this compound the initiation of development of normal ectodermal appendages such as sweat glands, teeth, hair, and sebaceous glands would fail. Step C must take place before step D can occur, and D before E; hence, failure of step C would result in absence of the end product, e.g., sweat glands, or partial impairment could result in malformation of the end product. Failure of initiation of the development of sweat and gland anlage could in turn cause a failure to produce the stimulus or milieu for the development of the sebaceous gland anlage. On the other hand, step C may be common to all skin appendages in embryogenesis. The principle involved is that tissue and organ differentiation and development are the result of sequential biochemical steps which are affected by both gene constitution and the surrounding cellular environment.

SUMMARY

1. A family has been studied whose members showed ectodermal dysplasia and corneal dystrophy (Groenouw type 1), either singly or in combination.

2. By carefully studying the total body surface for the capacity to sweat, a number of otherwise normal individuals have been shown to be affected.

3. By the techniques employed, ectodermal dysplasia was shown to be transmitted as a dominant gene and expressivity is sex-affected.

4. The corneal dysplasia was also transmitted as a dominant gene. This condition is neither sex-linked nor sex-affected.

ADDENDUM

Since the writing of this paper, Dr. George G. Graham, Cleveland Clinic, informs us that he has under his care a male infant with marked heat intolerance and an abnormal skin resistance pattern. The father has an abnormal skin resistance pattern, whereas that of the mother is normal. The paternal grandfather is said to have observed anhidrosis of one side of his face.

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EXANTHEM ASSOCIATED WITH ECHO VIRUS TYPE 18 VIREMIA

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RECENTLY probable etiologic relationships between certain human exanthematous diseases and some of the enteroviruses have been demonstrated.[1-3] Neva[4-7] has described the clinical and virologic characteristics of an illness presenting with a rash due to Echo virus type 16. Sabin[8] has reviewed Echo type 9 virus disease and the associated febrile exanthem has been described.[9, 10] In epidemics Echo viruses types 4, 9, and 16 have been associated with an exanthem; whereas in isolated cases Coxsackie virus type A9 and Echo viruses types 2, 6, and 14 have been so associated.[3] Enanthemas have also been reported.[3] Generally speaking, although these illnesses can be clinically differentiated from rubeola, rubella, and roseola infantum it is difficult to distinguish between the various exanthemas caused by enteroviruses.

Echo virus type 18 was first isolated by Ramos-Alvarez[11, 11a] during investigations of summer diarrhea. Since then, Eichenwald, and co-workers[12] have clearly shown it to be etiologically responsible for an epidemic of diarrhea in infancy. Until very recently the virus had been recovered only from stool specimens. Karzon[13] has now recovered the agent from the cerebrospinal fluid and stool of a patient who had aseptic meningitis without a rash.

The report of the following case and its associated virologic studies is made because the virus was isolated from the blood and it seems probable that the febrile exanthematous illness was caused by the recovered agent which was Echo virus type 18 or a closely related variant.

CASE REPORT

History.—S. D., JHH B 55600, a 3-week-old white girl, was admitted to the Harriet Lane Home, of the Johns Hopkins Hospital at 1:00 A.M. on September 25, 1958, because of apneic periods associated with a febrile illness.

She had been well until September 23, when her temperature was found to be 100.6° F. It had been taken because her 18-month-old brother was ill and had a temperature of 102° F. Throughout the night she was irritable but there were no other signs of illness. Because of irritability, lethargy, and persistence of fever, throat swabs for bacterial cultures were obtained from both children the next morning. Her temperature was then 102° F. During the afternoon preceding the night of admission, pharyngitis and a postnasal drainage were noted. The sibling concurrently had a rash which appeared after severe crying. The patient was given Terrabon, 10 Gtt. every 6 hours, Tempra, 0.3 ml. every 4 hours, and was sponged p.r.n. Late in the evening, during the course of an hour, she had four episodes of apnea

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and decreased muscular tone. Each lasted 15 to 20 seconds, and she was brought to the hospital.

The infant was born at Johns Hopkins Hospital on September 4, 1958, following an uneventful pregnancy. The infant did well and was discharged on the fourth day after birth.

A systems review was otherwise noncontributory. There were no gastrointestinal or other central nervous system symptoms. Neither was there contact with known contagious disease, although the family lived in a resident staff housing area through which various mild illnesses moved freely. "Summer" diarrhea was current.

The infant was a well-developed, well-nourished, white girl in no acute distress who became dusky during intensive crying. Her temperature was 101° F.; pulse, 128 per minute; respirations, 32 per minute; blood pressure, 120/70 (right arm, infant crying). Although the skin was mottled there was no rash. The tonsillar pillars were injected but there was no exudate or characteristic enanthem. A watery yellow stool was expelled during the examination. The examination was otherwise normal.

Laboratory Data.—Studies of the peripheral blood showed the following: hematocrit, 44; white blood cell count, 10,456 per cubic millimeter; differential, 32 polymorphonuclear cells, 12 segmented cells, 50 lymphocytes, 5 monocytes, and 1 metamyelocyte; red blood cells, normochromic and normocytic; many platelets.

The spinal fluid was a clear, colorless fluid, with normal hydrodynamics. Pandy was negative. Five tube semiquantitative sugar; 3 tubes positive (indicating a sugar content of approximately 60 mg. per cent). There were 229 fresh red blood cells and 3 lymphocytes per cubic millimeter.

An analysis of the urine was normal. Roentgenograms of the chest were normal.

Cultures.—A throat culture taken on September 24, 1958, revealed a heavy growth of normal flora; *Staphylococcus albus* was the predominating organism found. On September 25 the following cultures were taken: nasopharynx, diphtheroids and hemolytic *Staphylococcus albus;* throat, heavy growth of hemolytic *Staphylococcus albus;* stool, no enteric pathogens; cerebrospinal fluid, no growth; blood, no growth.

Hospital Course.—The infant was given Terramycin, 40 mg. every 6 hours per os. No Tempra was given. She had no more apneic periods and her general condition was good. Her fever persisted however. Her temperature was 102° F. when a generalized erythematous macular rash which appeared blotchy and could be blanched by pressure was first noted in the evening of September 25. No petechiae, enanthem, conjunctivitis, or adenopathy were noted.

Eleven stools were passed during the first 24 hours of the hospital stay, 3 to 4 the next 2 days. These were not considered diarrheal in nature. The Terramycin was discontinued on September 26.

Her fever then began to subside. On the following day the rash was intensely erythematous and generally macular but some areas were slightly papular. It blanched on pressure, was most intense and extensive on the face, but involved all areas of the skin including the palms and soles. The blotches were more discrete than coalescent. The mouth and pharynx were normal. Small movable, nontender, barely palpable occipital and posterior cervical lymph nodes were found. Although somnolent, the infant was otherwise not unusual. Throat and anal swabs were obtained September 27 for viral cultures and the infant was then discharged. The rash gradually faded during the next 48 to 72 hours without desquamation. The infant's subsequent course was uneventful. Convalescent serum was obtained October 29.

The sibling's illness was very similar. He had no apneic spells, his temperature reached 105° F., and his rash appeared prior to Terrabon therapy. He had, however, received 1 or

2 doses of Tempra. He was not studied virologically. The parents were asymptomatic. A blood specimen was obtained from the mother on October 29.

METHODS OF INVESTIGATION

Throat and anal swabs were separately immersed in 2 ml. of Hank's balanced salt solution containing antibiotics. Swabs were squeezed thoroughly, the resultant suspensions were centrifuged, and the supernatants stored in screw-capped vials at –50° C. The serum and clot of an acute blood specimen obtained from the patient were separated and stored at –50° C. Only the sera of the patient's convalescent stage and that of the mother's blood were saved.

Monkey (cynomolgus and rhesus) renal epithelial cell cultures were grown in the usual manner[14] but maintained on skimmed milk maintenance medium.[15] Serially passed, trypsinized human embryonic skin and muscle cultures were employed only to determine the effect of the throat isolate on them. Cultures were rinsed before being inoculated with 0.1 or 0.3 ml. of the material to be tested. They were then rolled at 37° C. and regularly observed for cytopathic effects. The blood clot was ground with alundum and an equal quantity of Hank's balanced salt solution; this was centrifuged, and 0.1 and 0.3 ml. of the supernatant inoculated. The blood was handled entirely apart from all other specimens.

Viral suspensions, either original specimens or prepared pools, were titered and the TCD_{50} per milliliter calculated according to accepted methods.[14]

Recovered cytopathogenic agents were identified by neutralization tests performed in tissue culture, and serum neutralizing antibodies were quantitated by the same means. These neutralization tests were carried out using pools of the recovered agents which represented their fourth monkey kidney passage and contained about 10^6 TCD_{50} per milliliter. Aliquots of such pools were stored in ampules in a CO_2 chest. The methods recommended by the Committee on Enteroviruses[16, 17] were employed, except as follows: in every instance Hank's balanced salt solution was used as diluent for virus and for serum. Each culture received an 0.2 ml. inoculum containing 30 to 400 TCD_{50}* after equal quantities of the viral suspension and the serum dilution had been mixed and incubated for 90 minutes at 4° C. All mixtures were tested in duplicate. Concurrent titrations checked the quantity of virus actually employed. Daily observations revealed the apparent antibody titer in terms of that dilution of serum which completely prevented the appearance of cytopathic change.

Goldfield and his associates'[18] method for determining enterovirus hemagglutinin was used except that the virus-red cell suspension was allowed to sediment at 4° C. and human red blood cells, type A, were used. In our hands other sedimentation temperatures gave much lower titers. A prototype strain of Echo virus type 11 was used as a known hemagglutinating enterovirus.

Plaques were produced on monolayers of rhesus monkey kidney epithelium by the method of Hsiung and Melnick.[19]

Suckling mice less than 1 day old were inoculated intracerebrally with 0.02 ml. and intraperitoneally with

*An attempt was made in each instance to use 100 TCD_{50}.

0.03 ml. of undiluted tissue culture fluid which contained $10^6 TCD_{50}$ per milliliter of the fourth monkey kidney passage of the cytopathic agent recovered from the patient's blood clot. The mice were observed for 21 days. A suspension made from whole carcass mince of dead mice was similarly passed in the mice.

A rabbit was hyperimmunized with fourth passage material of the cytopathic agent recovered from the patient's blood. One milliliter of undiluted tissue culture fluid containing $10^6 TCD_{50}$ per milliliter was inoculated intravenously at weekly intervals over a period of 5 weeks. Three milliliters were inoculated intramuscularly at two sites and the procedure repeated after one month. An adjuvant was not used. Bleedings were begun 5 weeks after the first injection.

RESULTS OF VIROLOGIC INVESTIGATIONS

Cultures inoculated with 0.3 ml. of the throat and anal swabs became positive for viral cytopathogenic effect (CPE) on the sixteenth and sixth days postinoculation, respectively. Those inoculated with 0.1 ml. of the material from the blood clot became positive on the thirteenth and the twentieth days; isolations were made on two occasions. In addition, Dr. Franklin A. Neva[20] recovered a cytopathogenic agent in human embryonic skin and muscle from an aliquot of the blood clot material. The agent recovered from the throat swab (Dejter throat agent) was passed twice in human embryonic skin and muscle. It rapidly destroyed the cell sheet and had a titer greater than 10^3 TCD_{50} per milliliter. Titration of the original specimens after storage revealed that they contained 10 to 30 TCD_{50} per milliliter.

The results of various neutralization tests are tabulated in Table I. The cytopathogenic agent recovered from the patient's blood, the Dejter blood agent (DBA), was neutralized only by rabbit antiserum prepared against Echo virus type 18 and by the patient's convalescent serum. Antisera prepared against all other enteroviruses which characteristically produce a viral cytopathogenic effect in monkey kidney were tested and none significantly inhibited the Dejter blood agent. Dr. Neva[20] tested the DBA against Echo 4 and 16 monkey antisera; there was no neutralization. Results of cross-neutralization tests also show that the DBA is an Echo 18. In addition to the neutralization of DBA effected by commercially available Echo 18 rabbit antiserum, serum from a rabbit immunized with DBA contained significant neutralizing antibody for Echo 18. The patient's convalescent serum similarly contained antibody for both DBA and Echo 18. In every instance, however, serum could be more highly diluted and retain its neutralizing capacity against prototype Echo 18. These apparent discrepancies in titer could be due to different quantities of virus employed, but it is more likely that this is a demonstration of the variation in antibody avidity possessed by different strains of Echo virus types. This phenomenon, similar to the P-Q-R phases of influenza viruses, has been studied by Karzon[21] and by Neva.[20]

Neutralization of the cytopathic effects of the Dejter throat and rectal agents by antiserum against prototype

Echo virus type 18 and the Dejter blood agent indicate, of course, that these too are Echo 18 viruses.

produced on monolayers of monkey kidney epithelium by the Dejter blood agent.

TABLE I. RESULTS OF NEUTRALIZATION TESTS

VIRAL AGENT	SERUM							
	PATIENT DEJTER		RABBIT HYPER-IMMUNIZED WITH DBA		ANTI-ECHO 18	NORMAL RABBIT SERUM	PATIENT'S MOTHER	ANTISERA: COXSACKIE A9, COXSACKIE B1-5, POLIO VIRUS 1-3, ECHO VIRUSES 1-17, 19-24
	ACUTE	CONVALESCENT	PRE	POST				
Dejter blood agent (DBA)	<2①/②	256/16	<4/—	8/4	180/90	<5/—	4/2	No neutralization
Echo 18 prototype	ND	64/32	ND	32/16	720/720	ND	8③/2	ND
Dejter throat agent	ND	ND	ND	10④/<10	45④/⑤	<4/—	ND	Coxsackie A9 and Echo viruses 2, 4, 6, 9, and 14: No neutralization
Dejter rectal agent	ND	ND	ND	10④/<10	45④/45	<4/—	ND	No neutralization

① Reciprocal of highest serum dilution which completely prevented a viral cytopathogenic effect as determined on second day.

② Same as ①, but as determined on fifth day.

③ Only serum dilutions 1:2 and 1:8 were tested.

④ Only serum dilution tested.

⑤ On fifth day one tube had a viral cytopathogenic effect, the other did not.

ND = Not done.

During the acute phase of her illness, the patient had no demonstrable circulating neutralizing antibody. After convalescence, however, the serum could be diluted 1:256 and it would effectively neutralize the Dejter blood agent's cytopathogenic effect for a short, but significant, period of time. This demonstrates a temporal association of the Echo 18 infection and the exanthematous illness.

The Dejter blood agent did not hemagglutinate human red blood cells even when diluted only 1:2. The dilution was prepared from a suspension containing $10^{6.5}$ TCD_{50} per milliliter. The virus did not produce overt disease in suckling mice. Two of a litter of 8 mice died within 24 hours, but material from these had no demonstrable effect upon passage in suckling mice. Small irregular plaques were

DISCUSSION

The results of these virologic investigations clearly show that this infant was infected with Echo virus type 18 at the same time that she had a febrile exanthematous disease. Cytopathogenic viral agents recovered from her blood, throat, and rectum were neutralized by Echo 18 antiserum. During convalescence she developed a significant rise in titer of serum neutralizing antibody against the virus isolated from her.

Whether her exanthem was caused by this concurrent viral infection cannot, however, be absolutely determined. She had received acetaminophen and oxytetracycline prior to the appearance of the rash. Both medications, or chemically very similar ones, have been associated with, and thus

presumably have been the cause of morbilliform skin eruptions.[22, 22a] The clinical features of such rashes are not sufficiently characteristic to permit an etiologic diagnosis.[23] She has not received either drug since nor is there any history of sensitivity or previous experience with them. Because her sibling concurrently had a very similar illness, it seems probable that the illnesses were due to a common infectious etiology rather than to infection plus drug eruption.

Echo 18 infections have been previously associated with diarrheal disease. Neither child had diarrhea sufficient to arouse concern, but diarrhea was present among other children in the housing development. The infant's frequent stools began after oxytetracycline was begun. From 3 infants living in the surrounding neighborhood who were ill at the same time with similar febrile rash diseases, cytopathogenic agents have been recovered. One was Echo 18. Studies which will attempt to determine whether these were Echo 18 infections and whether they were then prevalent are now in progress. Only by discovery and description of other uncomplicated cases of Echo 18 exanthemas can this probable case be verified and the disease defined.

The clinical features of the known Echo virus exanthemas have been outlined by Sabin.[3] Some are associated with an enanthem. This patient had no enanthem. Her illness resembled Echo 16 (Boston-Pittsburgh) exanthem in that the rash appeared as the temperature fell. The febrile prerash period, however, was shorter than is usual with Echo 16 disease. Echo virus diseases tend to occur in family outbreaks, and adults often manifest their disease somewhat differently than do their children. For example, adults frequently have aseptic meningitis and children more frequently have a rash in epidemics of Echo 9 disease. This infant's parents, however, were asymptomatic and aseptic meningitis was not prevalent in the area. Beyond these meager points there is very little of differentiating value between the enterovirus exanthemas. St. Geme and co-workers[9] have emphasized this difficulty of clinically distinguishing between the various exanthematous Echo virus diseases.

Differentiation from the more common infectious morbilliform exanthemas is less difficult.[23] Both measles and exanthema subitum have longer and characteristic prodromal periods. The rash of exanthema subitum does not persist as long as did this one. The fever of rubella is usually much less than this child's. Her rash was also more intensely erythematous, extensively distributed, and more persistent than that of rubella.

SUMMARY AND CONCLUSION

A 3-week-old white girl had a febrile exanthematous disease temporally associated with Echo virus type 18 viremia. This viral agent was also recovered from her throat and rectal swabs. Between the acute and convalescent stages of her illness, she developed an 8-fold rise in the titer of neutralizing antibody. An 18-month-old sibling had a very similar exanthematous illness, but he was not studied. Although the rash may have been due to acetaminophen or oxytetracycline, this is probably the first reported case of Echo virus type 18

exanthem. It is the first reported isolation of this virus from the blood. Definition of this disease must await additional observations.

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INDICATIONS AND CONTRAINDICATIONS FOR SURGERY IN VENTRICULAR SEPTAL DEFECT

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THE dramatic advances in open heart surgery have led to a great increase in our knowledge of ventricular septal defect and the physiological and pathological changes involved. Now that closure of these defects is widely practiced, it behooves the pediatrician to know not only the possible benefits of surgery but also the possible risks; furthermore, it is important to understand the natural history of the patient if left unoperated. Although, as is well known, ventricular septal defects occur in combination with numerous other malformations of the heart, it is our present purpose to discuss only those patients in whom the defect occurs as an isolated malformation or in association with slight overriding of the aorta. Three main groups of patients are involved in the present discussion; namely, those with small ventricular septal defects and small shunts, those with moderate-sized or large defects and large left-to-right shunts, and those with cyanosis and a reversed or right-to-left shunt, that is, the so-called "Eisenmenger complex."

In the first group the left-to-right shunt is small and surgery is contraindicated because the natural history is so benign that the risks of surgery outweigh the possible benefits. In the large intermediate group with a large left-to-right shunt surgery is both practicable and of great potential benefit. The recognition of which patients fall into this group and the correct timing of their surgery is one of the most important tasks facing the pediatrician and cardiologist at the present time. Patients with shunt reversal and cyanosis or the "Eisenmenger complex" have a grave natural prognosis but the risks of surgery are at present prohibitive.

The general principles which should be used in selecting patients for cardiac surgery of any kind have been discussed previously.[1] In patients with a ventricular septal defect surgery is very rarely immediately lifesaving since natural death once infancy is passed is extremely rare.[2] Surgery is therefore undertaken in these patients with the idea that it will prolong life, render it more enjoyable and carefree, and cause a return of the pulmonary artery pressure and resistance to normal.

The size and position of the defect and the degree of overriding of the aorta determine to a large extent the volume and direction of the shunt, the severity of the clinical symptoms,

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and the nature and extent of the vascular changes in the lungs. In some instances the pulmonary vascular changes are more serious than the load placed on the heart by the defect. Because experience has shown that patients with far-advanced pulmonary vascular changes usually do not survive surgery, or if they do the pulmonary hypertension is not relieved, there is a widespread but we believe erroneous idea that all patients with ventricular septal defect must have an operation at the *earliest possible time* to prevent irreparable damage to the lungs. In order to determine which patients can be expected to benefit from surgery and those in whom surgery is contraindicated, we must review briefly our current understanding of the vascular changes which occur in the lungs of patients with ventricular defects.

The nature of the pulmonary vascular changes in ventricular septal defects has been extensively studied by Edwards[3] and by Dammann and Ferencz.[4] They have found that in infants with left-to-right shunts the pulmonary arterioles retain the medial hypertrophy characteristic of the fetal state for many months; consequently, a large number of the small arterioles remain as vessels with small lumina and thick muscular walls. The normal expansion of the pulmonary vascular bed is thus delayed; the thickened arterioles offer resistance to the flow of blood to the lungs and thereby prevent an excessive left-to-right shunt. Gradually, over a period of years the thickened vessels expand. Their lumina increase in size, and the muscular walls become thinner while the intimae remain smooth and delicate. As these vessels expand the resistance to the pulmonary blood flow is diminished and the pulmonary pressure falls. Such is the usual and indeed favorable sequence of events in patients with a small- or moderate-sized ventricular defect. Indeed, if an infant has a large left-to-right shunt and the pulmonary vascular bed expands rapidly in the normal fashion, the infant suffers from an excessively large shunt and may even develop pulmonary edema. The slow opening up of the pulmonary vascular bed may therefore be thought of as a protective phenomenon in that the failure of the smallest arterioles to expand at the normal rate acts to maintain the high pressure which is normal during fetal life and lessens the left-to-right shunt. This phenomenon in itself does not lead to progressive pulmonary hypertension; on the contrary, it is a protective phenomenon occurring mainly during the early years of life when the dangers of cardiac failure and pulmonary edema are at their greatest.

Although the immediate reaction of the lungs is the same in all infants with a large left-to-right shunt, Edwards has shown that the sequence of events differs when the right ventricle is pumping blood against systemic resistance. If the right ventricle is acting to maintain the systemic circulation, as in aortic atresia or in severe coaretation of the aorta with a large ductus distal to the coaretation, the long-continued ejection of blood to the lungs under high pressure leads to intimal proliferation and progressive injury to the pulmonary vascular bed with eventual

extreme arterial degeneration and even sometimes necrosis. Similar changes may occur if the defect between the two ventricles is so large that they funtion virtually as a single chamber and blood is ejected to the body and lungs under the same pressure. This has been termed by Dammann and Ferencz as "a common ejectile force" and eventually does lead to progressive vascular damage and irreversible changes in the pulmonary arterioles. Thus, the problem presents itself as to whether the child with pulmonary hypertension and a ventricular defect has primarily a protective pulmonary hypertension with medial hypertrophy and slight intimal proliferation and early fibrosis only, with a basically normal pulmonary vascular bed, or whether there are also those forces at work which lead to progressive intimal thickening and irreversible and far-advanced vascular damage. Indeed, the extent of injury to the lungs in a patient with a ventricular septal defect and the severity and possible reversibility of the associated pulmonary hypertension are the most serious and challenging problems which confront the cardiologist in the estimation of the risk of surgery and the amount of benefit which the patient may derive therefrom.

Certain it is that the lower the pulmonary pressure the more normal is the lung. Indeed, if pressure is normal, there is no danger of the development of pulmonary hypertension. Furthermore, if the pressure is moderately elevated but not at systemic levels, there is not a systemic right ventricle or a "common ejectile force," and the patient is not in danger of developing severe or progressive pulmonary hypertension in adolescent life. Nevertheless, over a long period of time a large pulmonary blood flow and some elevation of pulmonary artery pressure does lead to some degree of intimal change and surgery is indicated, although it is not highly urgent, in patients with moderately elevated pulmonary artery pressure during their childhood years.

In the light of these findings one may consider the various types of ventricular septal defect.

GROUP I. SMALL VENTRICULAR SEPTAL DEFECT: "MALADIE DE ROGER"

From the above discussion it is clear that small ventricular septal defects do not injure the lungs. They are compatible with a long and active life and surgery is contraindicated at present because the possible benefits are outweighed by the risks involved.

Clinically these patients are asymptomatic or become dyspneic only after strenuous sports. Growth and development are usually normal. The parents frequently state they "can hardly believe there is anything wrong" with the child. The cardiac murmur is usually loud and rasping and there is a good thrill; the pulmonary second sound is little if at all increased and there may be a short apical mid-diastolic rumble. The size of the heart may appear slightly increased in infancy but later is within normal limits and the electrocardiogram shows minimal T-wave changes over the left precordium. Cardiac catheterization confirms that the left-to-right shunt is small and the pulmonary flow only about twice systemic,

while the pulmonary artery pressure and resistance is at or near normal levels.

Surgery in these patients cannot improve their already excellent clinical condition. There is no evidence that they will develop pulmonary hypertension later; hence, surgery is not indicated on that account. Therefore, these patients should not be submitted to an operation unless it can be proved that the operation results in such perfect closure of the defect as to eliminate the danger of subacute bacterial endocarditis and that the operative risk is negligible. Although these small defects are easily closed, the operative risk of transfusion reaction, of injury to the bundle of His, and the development of complete heart block and the risk of endocarditis in the immediate postoperative period remains, as do the unknown long-term complications of ventriculotomy itself. In some instances the defect has reopened postoperatively and the patient has been more incapacitated than before. These risks are too great to justify surgery to prevent subacute bacterial endocarditis, especially as our ability to treat this disease successfully is continually improving.

GROUP II. MODERATE OR LARGE VENTRICULAR SEPTAL DEFECT: "OPERABLE"

This heterogeneous and fascinating group includes patients with defects of varying size and location. It includes patients with minimal symptoms and some in chronic cardiac failure; it includes those with mild left ventricular hypertrophy and others having extreme degrees of combined ventricular hypertrophy. Common to the whole group, however, is the left-to-right shunt of sufficient magnitude to warrant closure and a reversible elevation of pulmonary artery pressure and resistance.

Patients With Mild Pulmonary Hypertension.—These patients show mild to moderate growth retardation, slightly increased susceptibility to infection, and a slight exercise limitation. The cardiac murmur and thrill are distinct and the pulmonary second sound is somewhat accentuated. Clinically and radiologically there is slight cardiac enlargement and there is left ventricular hypertrophy. The left atrium is enlarged and there may be slight right ventricular hypertrophy. Cardiac catheterization confirms the fluoroscopic impression of greatly increased pulmonary flow; the pulmonary artery systolic pressure is elevated to between 40 to 60 per cent of systemic levels while the pulmonary diastolic pressure is only slightly elevated and the pulmonary resistance may indeed be less than normal.[5]

Operation is not urgent for these children since their pulmonary hypertension is not progressive during the childhood years. However, they can be benefited and surgery is justified if a very low mortality rate can be maintained.

Patients With Moderate or Severe Pulmonary Hypertension.—Patients in this group may have a very stormy infancy, especially if the defect is either very large or is associated with an overriding aorta. Repeated respiratory infections, severe vomiting and feeding difficulty, and even frank congestive failure may occur in the first year of life. Digitalization,

prophylactic antibiotics, and incessant watchfulness may be needed. As the children grow the problems recede; the children usually remain small and underweight and respiratory infections are still frequent but less severe. Dyspnea becomes less marked but there is always some reduction of exercise tolerance, although it is not always recognized by the parents until after successful surgery has been performed. Cyanosis may be present during failure or in infancy on prolonged crying but is usually absent at rest and in older children.

The cardiac murmurs in those with severe pulmonary hypertension are very variable, especially in early childhood. Indeed, at times, especially during periods of infection or failure, the murmur may be blowing and diffuse and lead to diagnostic error: although not all patients with severe pulmonary hypertension show softening and shortening of the systolic murmur, this sign, when present, indicates that pulmonary and systemic pressures are approximately equal. A rumbling apical mid-diastolic murmur is very frequent. The pulmonary second sound is accentuated and sometimes palpable. Cardiomegaly is apparent clinically and radiologically. The left ventricle is invariably hypertrophied and the left atrium enlarged; the right ventricle shows varying degrees of hypertrophy. In some instances the right ventricular hypertrophy is so great that enlargement of the left ventricle may be overlooked. DuShane and also McNamara and Latson have emphasized that the relative significance of the defect itself and of the pulmonary vascular change is reflected in the amount of work required of the two ventricles. If the left atrium and left ventricle are bearing the major strain, the risk of operation is far less than when the work of the right ventricle overshadows that of the left. Therefore, evidence of left atrial and left ventricular enlargement is sought for assiduously in patients with ventricular septal defect and pulmonary hypertension and may be found either clinically, radiologically, or on the electrocardiogram. Cardiac catheterization confirms the impression that the pulmonary flow is greater than the systemic, even in some cases eight times greater: both the systolic and diastolic pressures in the pulmonary artery are elevated and the systolic pressure may often be equal in the two circulations. The pulmonary resistance is elevated and may reach two thirds of the systemic resistance in infants or in some children with severe pulmonary vascular changes.

Great advances have been made in our understanding of these patients with ventricular septal defect and severe pulmonary hypertension in the past few years because of the extensive physiologic studies which have been performed and also because of the successful operations in this group. Studies at the Mayo Clinic[6] and also by McNamara, Latson, and Cooley[5] have shown that the pulmonary resistance in most of these patients is "labile," that is, it falls, although not necessarily to normal levels, on inhalation of 100 per cent oxygen. The labile nature of the pulmonary hypertension is confirmed when the pulmonary artery

pressure falls toward normal immediately following surgery and it often reaches normal levels a few months later. Recent studies by Heath and Edwards[7] have shown that where only medial hypertrophy or mild intimal proliferation is present in the pulmonary arterioles the pulmonary resistance is more labile than when intimal fibrosis and vascular dilatation have appeared; the latter changes do not, however, preclude a successful operative result. Although in these patients a right-to-left shunt of small volume may be present, it is far less than the left-to-right shunt, and furthermore, it can usually be abolished by the inhalation of oxygen.[5]

The patients with moderate-sized defects tend to show a drop in the pulmonary artery pressure once infancy is past, which corresponds to the gradual opening up of the pulmonary vascular bed previously discussed. For such patients there is no urgency for surgery. In patients with very large defects whether or not associated with overriding of the aorta there are probably two processes going on simultaneously. There is some regression of the infantile pattern of medial hypertrophy which causes a slight *decrease* in pulmonary resistance, and at the same time the continued high ejectile pressure in the lungs causes some intimal damage and leads to intimal fibrosis and arteriolar dilatation with an *increase* in pulmonary resistance. Usually, these two tendencies act to counterbalance each other for a number of years and the pulmonary artery pressure and resistance remain at fairly constant levels throughout childhood. Those patients with very large defects or with an overriding aorta, that is, those with a true "common ejectile force," are in danger of progressive damage to the lungs. Therefore, surgery is better undertaken in early childhood in this group.

This whole group of patients with moderate or large left-to-right shunts and increased pulmonary artery pressure needs surgery and may be greatly benefited thereby. The risk of operating in infancy is at present undoubtedly great and usually it is not necessary. However, the small puny infant with almost no weight gain and recurrent attacks of combined "pneumonia" and pulmonary edema poses a special problem. In most instances medical management still offers the best hope, but occasional dramatic results may be obtained from open heart surgery or from "banding" procedures. The medical management of these small sick infants is a challenge to the skill of the pediatrician. It is his responsibility to help the infant survive the numerous crises of the early months until the best age for surgery is reached.

GROUP III. "EISENMENGER TYPE"

The classical conception of this syndrome is well known, namely, poor weight gain, respiratory infections, and feeding difficulty in infancy, followed by a comparatively normal later childhood with slightly reduced exercise tolerance and the gradual onset of cyanosis in adolescent life. Pulmonary hypertension has been present from birth but during the third and fourth decades of life the

complications of pulmonary hypertension, namely, severe dyspnea, chest pain, hemoptyses and right-sided cardiac failure, develop. There is much evidence, as we have seen previously, that this progression of symptoms only occurs if the defect is very large or if there is significant overriding of the aorta, and even in this group it is by no means the invariable course of events. It is certain that the above sequence of events does not represent the natural history of *all* ventricular septal defects. By the time the lung damage is so severe that pulmonary hypertension is irreversible, the patient usually shows slight persistent cyanosis, is dyspneic on mild exertion, and has the physical signs of pulmonary hypertension, namely, a loud palpable pulmonary second sound, a right ventricular outflow lift, a prominent "A" wave, and a variable, sometimes insignificant systolic murmur. There is usually little cardiac enlargement and as evidenced by x-ray and electrocardiogram the enlargement is of the right atrium and right ventricle only. Cardiac catheterization confirms the fluoroscopic impression that the pulmonary flow is little if at all increased and shows a right-to-left shunt equal to or greater than the left to right. The pulmonary resistance is elevated to near systemic levels and shows little change on breathing 100 per cent oxygen.[5, 6] The pulmonary resistance is then described as being "fixed."

Patients in this physiologic state are inoperable at the present time; for even if they survive the immediate postoperative period there is usually no fall in the pulmonary artery pressure. Heath and Edwards and associates[7, 8] have shown that such "irreversible" pulmonary hypertension is associated with the most severe pulmonary vascular changes, including necrotizing arteritis. It is in an effort to avoid the development of this late stage that operation is recommended in early childhood in patients with very large defects or with an overriding aorta.

There is another group of patients in whom even from infancy pulmonary hypertension seems to be more important than the ventricular defect. These children show recurrent cyanotic attacks from early infancy and often faint persistent cyanosis from a very early age: chest pain and syncope may occur in young childhood. The nature and degree of pulmonary vascular change is obviously more malignant than in the more typical Eisenmenger complex, but whether it represents a completely different process is by no means clear. In these patients the pulmonary hypertension appears to be primary and the ventricular septal defect is incidental. Successful treatment of these patients will come as our knowledge of the cause of pulmonary hypertension increases.

SUMMARY AND CONCLUSIONS

Patients with small ventricular septal defects and small left-to-right shunts, the so-called "Maladie de Roger" group, should not be operated on at the present time because there is no risk of the development of pulmonary hypertension and because the risks of surgery outweigh any possible benefits.

Patients with mild or moderate pulmonary hypertension show a steady level of pulmonary artery pressure during the childhood years and in many instances the pulmonary artery pressure falls as the years pass. This is in contrast to the commonly held idea that pulmonary hypertension is steadily progressive throughout childhood and makes surgery a matter of urgency in all children with ventricular septal defect. These children can be greatly benefited and surgery is indicated for them if the operative risk can be kept at minimal levels.

Children with a large left-to-right shunt and severe pulmonary hypertension are the major problem at the present time, both in management during infancy and in selection of the best time for operation. Although operation in infancy remains dangerous, it may in rare instances be justified and certainly operation in early childhood is to be considered in those children with severe right ventricular hypertrophy and a sufficiently large defect in order to ensure that blood is being ejected into the lungs at systemic levels of pressure or under a "common ejectile force."

Patients with shunt reversal, cyanosis, fixed pulmonary resistance, and a predominant right-to-left rather than left-to-right shunt are classified as the "Eisenmenger" complex and are not candidates for surgery until our understanding of the postoperative management of pulmonary hypertension increases.

The selection of patients for open heart surgery is now firmly based on physiologic studies and on our recent increased understanding of the importance of the pulmonary vascular bed.

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HEARING RESPONSES AND AUDIOLOGIC SCREENING IN INFANTS

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THE work presented in this paper came about as the result of a demonstration of auditory screening methods applied to infants 7 to 13 months of age by Dr. and Mrs. Ewing[9, 10] from the University of Manchester, England. We were impressed with the possible public health and research application of these techniques. It seemed very probable that they could be adapted for use with infants younger than 7 months of age.

During the past decade the practicality and value of audiologic screening procedures for school-age children has become widely recognized. Regular audiologic screening has become an important case finding tool in the well-organized school health program. On the basis of failure to perform the screening test satisfactorily on two successive occasions, a week or two apart, the child in need of definitive audiologic evaluation (including an examination of the ears, nose, and throat) can be singled out. On the basis of a thorough evaluation, special medical and educational consideration may be given where indicated.

While the importance of such a program cannot be underestimated, particularly as regards the conductive type of hearing loss acquired during the school years, it is regrettable that many severe hearing problems go unrecognized until the child enters school. It is particularly regrettable when one realizes that speech and vocabulary are normally acquired at maximal speed during the second and third years of life. If the child with a communication problem is to be given the best opportunity to learn speech, the earlier the recognition and definition of his problem and the earlier special auditory and speech training is started the better.

Over the past 15 years satisfactory methods for the definitive study of preschool children with communication problems have been developed.[1-8] These methods, particularly the psychogalvanic method, are most satisfactory as diagnostic tools but require special facilities, highly trained personnel, and too much time to be practical for case finding.

When the Collaborative Cerebral Palsy Project was organized, under the sponsorship of the National Institute of Neurological Diseases and Blindness, arrangements were made by the

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This presentation is a preliminary report on work in progress as an ancillary study to the Collaborative Cerebral Palsy Project in the Johns Hopkins University and Hospital. The work is the result of joint thinking on the part of the group interested in "Communication Problems" at Hopkins. The technical work was done, for the most part, by Miss Anne Dougherty, with the assistance of Miss Evelyn Britt and Mrs. Judith Cohen, P.H.N. (since July, 1958).

Hopkins group to include auditory screening as one of the parameters to be studied.

The objective of the Collaborative Cerebral Palsy Project is the elucidation of eitologic factors responsible for cerebral palsy, mental retardation, congenital anomalies, and other neuropsychiatric defects, and the mechanisms through which these factors may operate. This is a prospective study requiring the intensive investigation of the mother during the prenatal period, labor and delivery, and of the child in the neonatal period. The child is then followed at intervals until he has entered school. Observations made during the follow-up of the child include careful pediatric, psychometric, neurologic, electroencephalographic, and other evaluations at suitable intervals. Abnormalities apparent in the child may be related back to events occurring during pregnancy, the perinatal period, or to events during childhood.

In order to investigate the effectiveness and practicality of audiometric screening of infants, one of the authors (A. D.) spent 2 months studying the techniques developed at the University of Manchester by Dr. and Mrs. Ewing.[9-10] Upon return, these techniques were adapted for use with younger infants. With the cooperation of Dr. Huntington Williams, Commissioner of Health of Baltimore City, a pilot study to test the validity of these techniques was set up in two child health clinics of the Eastern Health District, Baltimore City Health Department. In this pilot study 327 infants, 3 to 52 weeks of age, were tested. In addition, 111 infants 10 to 29 weeks of age have been tested in the follow-up clinic maintained as part of the Collaborative Cerebral Palsy Project Study.

The study of auditory responses has been extended to include newborn infants in the nurseries of the Johns Hopkins Hospital. These are infants in the Cerebral Palsy Project.

METHOD: INFANTS 3 TO 52 WEEKS

The auditory screening test used for infants 30 to 52 weeks of age was essentially that devised by the Ewings[9, 10] for infants 7 to 15 months of age. A modification of the Ewing test was used for infants 3 to 30 weeks of age. The Ewing test depends upon the child's interest in, and ability to respond to, sounds which vary in frequency characteristics and intensity within the general speech range.

The sound stimuli used are simple, inexpensive, effective, and reproduceable. They are calibrated with a sound meter within a fairly broad tolerance as to frequency and intensity. The following are the stimuli: (1) high frequency range, 40 decibels (db): high rattle, tissue paper, unvoiced consonants sss and kkk; (2) mid-frequency range, 40 db: middle rattle, cup and spoon, voice; (3) low-frequency range, 40 db: low rattle, voice, xylophone, tonette; (4) broad-frequency range, 2,000 to 8,000 kilocycles: squeaker, 50 db, clacker and bell, approximately 60 db.

The tests were made in a reasonably quiet room, equipped with a heavy carpet and curtains. The testing equipment was placed on a table behind the child. The table top was covered with a foam rubber mat. A chair for the mother and baby was placed

before the low chair occupied by the observer-reporter (Fig. 1). Care was taken to arrange the furniture in such a manner that shadows did not provide visual clues. Several attractive and colorful stuffed toys were used to engage the child's attention.

Fig. 1.—An auditory screening test on a 4-month-old infant in a clinic setting. The observer-reporter in front of the child engages his attention with a toy. The tester is using a china cup and spoon behind and to the right of the child. Some of the test equipment may be seen behind the mother.

An assessment of the child's general developmental status and present physical condition was made before each test. A brief pertinent medical history was obtained.

The technique used in auditory screening is referred to as the distracting technique (Fig. 2). The baby sitting in the mother's lap was supported under the arms and held away from the mother's body if necessary. This position enabled the child to move freely. The observer-reporter engaged and held the visual attention of the child forward by use of a toy. There was no verbal communication between the observer and the child or visual communication between the tester and the observer. The tester distracted the child by presenting an auditory stimulus out of range of the child's peripheral vision.

The stimuli used produce sounds that, generally, are meaningful to children. The sounds must be presented in an interesting manner. Voice sounds should be inviting and the unvoiced consonants delivered in a rhythmic fashion. The cup and spoon (Fig. 2) are used to make a stirring sound suggestive of food being prepared. The choice of the stimulus to be presented is governed by the tester's knowledge of its acoustic value. The effectiveness of the test is influenced by the agility of the tester and her knowledge of principles basic to the

test. Usually, less than 2 minutes are needed to complete a test in the age group 30 to 52 weeks.

In the absence of response the tester repeated the presentation of a particular stimulus at her discretion or selected a stimulus of comparable acoustic value. It must be kept in mind that the failure of the child to respond to a sound does not always indicate that the child did not hear the sound. The simple fact that he may not be interested in that particular sound can account for his lack of response. For this reason more than one stimulus with the same frequency characteristic is available for use.

Responses given by the children aged 3 to 30 weeks were recorded on a form specifically designed for this group. The stimulus, distance, intensity, and response were noted. If the stimulus was presented bilaterally and the responses varied, the more mature response was accepted. A head turn toward the source of sound was the only response recorded for the children in the 30 to 52 week age group.

It was noted on a number of occasions that upper respiratory infection prevented a satisfactory response from the child on either one or both sides during the period of infection. Once the infection subsided the child performed normally.

Fig. 2.—The clacker, bell, and tonette used in auditory screening for newborn and older infants.

RESULTS: INFANTS 3 TO 52 WEEKS

Effectiveness of Stimuli Employed.—The effectiveness of 12 different stimuli in the high, middle, and low frequency ranges in producing a response was tested in most of the infants. In some instances not all stimuli were applied because the child was tired or became otherwise uncooperative. The stimuli used and the responses they provoked are listed in Table I for children between 3 and 14 weeks inclusive and in Table II for children 15 to 30 weeks of age. It will be noted that some

stimuli were more effective than others and that the British stand-bys (the cup and spoon and the tissue paper) proved less effective in our setting than most of the other stimuli presented.

Hearing Responses Observed.—One of the objectives of these studies was to determine the type and the frequency of the responses observed following the various stimuli used. The

TABLE I. RESPONSES TO SOUND STIMULI EXHIBITED BY 130 INFANTS 3 TO 14 WEEKS OF AGE

TYPE OF STIMULUS	POSITIVE HEARING RESPONSE (PER CENT)				NO RESPONSE (PER CENT)	TOTAL NUMBER TESTED (100 PER CENT)
	HEAD TURN	EYE RESPONSE	MORO	OTHER		
Clacker	4	6	86	4	6	127
Bell	16	14	33	15	20	129
Tonette	19	32	18	18	14	74
Xylophone	16	38	16	18	12	114
Squeaker	16	26	36	12	11	101
Tissue paper	18	21	6	15	41	66
Cup and spoon	29	23	5	15	30	87
Low rattle	34	26	3	13	24	62
Middle rattle	31	28	11	18	12	121
High rattle	23	25	10	15	27	48
Unvoiced consonants	30	28	7	18	17	87
Voice	39	26	4	14	16	118

TABLE II. RESPONSES TO SOUND STIMULUS EXHIBITED BY 218 INFANTS 15 TO 30 WEEKS OF AGE

TYPE OF STIMULUS	POSITIVE HEARING RESPONSE (PER CENT)				NO RESPONSE (PER CENT)	TOTAL NUMBER TESTED (100 PER CENT)
	HEAD TURN	EYE RESPONSE	MORO	OTHER		
Clacker	11	6	65	3	11	212
Bell	38	27	23	4	16	217
Tonette	53	29	4	1	14	176
Xylophone	59	20	5	5	11	201
Squeaker	61	25	14	2	0.5	184
Tissue paper	61	14	2	1	22	194
Cup and spoon	61	16	1	0.5	21	183
Low rattle	63	8	1	0	27	100
Middle rattle	66	18	1	1	14	212
High rattle	54	16	1	0.7	21	134
Unvoiced consonants	71	16	0	0	13	197
Voice	76	12	2	1	3	216

On the basis of these findings it has been possible to streamline the test to the following items presented at approximately 40 decibels: (1) unvoiced consonants (high-frequency range), (2) middle rattle, and (3) voice (low range). If the child fails to respond, additional stimuli are offered. It thus becomes possible to screen the child, unless he is crying hard, in a matter of approximately 2 minutes.

following are the responses observed: (1) Moro reflex; (2) jumping and/or crying; (3) eye blink only; (4) eye blink and other responses; (5) grimace, frown or smile only; (6) general muscular movement; (7) immobilization; (8) disturbance of sleep; (9) eye opening, eye widening, and/or looking at observer; (10) eye turn and other responses; (11) searching; (12)

eye turn only; (13) head turn and other responses; (14) head turn only; (15) cessation of crying.

For the purpose of summarization, the responses have been grouped under the headings "head turn, eye response (excluding blink), Moro, and other." Head turn (of 45 degrees or greater) also includes head turn plus other responses. Eye response includes eye turn plus other responses, eye widening and searching movements of the eyes. An eye blink, seen quite frequently in younger infants, is included under "Moro" response. Moro response also includes a complete or partial Moro reflex, a body jump, or general muscular activity. Included under the category "other" are such things as cessation of crying, immobilization, and disturbance of sleep.

Because the type of response varied to some extent, both with the type of stimulus used and with the age of the child tested, the observations made are summarized in Table I for children of 3 to 14 weeks inclusive and in Table II for children 15 to 30 weeks of age. It will be noted that the predominant response in the younger group, particularly to fairly loud stimuli, is the Moro or partial Moro response. However, even in this developmentally rather immature group a significant number of head turns and eye responses were observed. In the group of infants between 15 and 30 weeks of age the Moro response becomes infrequent except to the very strong stimuli and the head turn and the eye response are predominant. As children between 31 and 52 weeks of age should be mature enough to respond with a head turn of 45 degrees, it was the required response for the group.

SUMMARY OF FINDINGS: INFANTS 3 TO 52 WEEKS

The results obtained when 438 infants, 3 to 52 weeks of age, were tested in a well-baby clinic setting are summarized in Table III. Of the 438 children, 96 were white and 342 were Negro. Detailed analysis of the responses exhibited by children of both races at comparable age levels failed to show any significant difference between the two races in responses obtained to the various stimuli presented.

TABLE III. HEARING SCREENING STUDIES: SUMMARY OF RESULTS IN 438 BABIES 3 TO 52 WEEKS OF AGE

GROUP TESTED	RACE		AGE IN WEEKS		TOTALS
	WHITE	NEGRO	3-30	31-52	
Eastern Health District					
Number tested	66	261	257	70	327
(Failed)	(1)	(18)	(15)	(4)	(19)
Washington Street					
Number tested	30	81	91	20	111
(Failed)	(4)	(6)	(10)	(0)	(10)
Totals	96	342	348	90	438

It will be noted that 19 of the 327 children tested in the clinics of the Eastern Health District failed to give a satisfactory response to the test. Of the 19, 15 gave good responses at the first retest, two at the second retest, and one at the third retest. The remaining child gave no response at the third retest and was referred for more definitive evaluation to the Johns Hopkins Speech and Hearing Center at 5

months of age. There he also failed to perform satisfactorily and was thought to have a "central communicative" problem. Subsequent investigation revealed that this Negro boy was completely unwanted and neglected, receiving virtually no stimulation and little care beyond feeding and occasional changing. Shortly after his visit to the Speech and Hearing Center he was "adopted" by a neighbor who gave him maximal care and verbal stimulation. When seen again at the Speech and Hearing Center at 9 months of age and again at 12 months, his performance was essentially normal. It is interesting to speculate upon the fate of his communicative skills if his state of auditory and other types of deprivation had continued.

Of the 111 children in the Washington Street group, 10 have failed the test. These children are being studied further either in the Hearing Research Laboratory at Washington Street or in the Johns Hopkins Speech and Hearing Center.

It should be pointed out that while auditory screening techniques are primarily directed toward determining whether the child hears, they also may serve as a rough screening for the developmental level of the child. The child who fails to produce a satisfactory head turn at 8 months of age may have a hearing problem or he may have a developmental problem and lack the maturity to perform in the desired manner, giving instead a more primitive or less mature response such as a Moro response. We have encountered a small number of children who are suspected of being developmentally retarded who respond poorly to voice sounds but who give more adequate responses to musical stimuli. This situation will be clarified as the children in the Cerebral Palsy Study will have a developmental evaluation by a psychologist at 8 months of age.

METHOD: THE NEWBORN INFANT

Group I.—Twenty-five ostensibly normal full-term infants 4 hours to 5 days of age and 18 premature infants 7 days to 2½ months old were studied in an effort to develop a satisfactory instrument for eliciting a hearing response.

A Moro or startle response or any part of the Moro response, such as a jerk or an eye blink, was considered satisfactory. In order that the observer could be sure that the response observed resulted from the stimulus applied, a response is considered positive only when it is elicited immediately following the stimulus two out of three times, the stimulus being applied at approximately 2 minute intervals. The stimulus is applied when the baby is awake. Three different instruments were tried, a clacker, a bell, and a tonette (Fig. 2).

Clacker: This is constructed of 2½ inch wood stock: one piece, 20 inches long, is the base; a second piece, 6 inches long, is screwed flush with, and end to end with, the long piece; a third piece, 11 inches long, extends from the 6 inch piece to which it is fastened by a 3 inch screen-door spring. When this latter piece is raised to a 90 degree angle and the whole apparatus held away from the body, the clacker is "cocked" for test use. This has a broad frequency range (up to 8,000 cycles) but with no distinct harmonics. In contrast with the bell its sound has a very short duration (perhaps 5 milliseconds).

*Doorbell**: This is a standard 3 inch bell, mounted on a 2 by 8 inch board and connected to a standard 6 volt dry cell battery to which the board is taped. It has a wide-frequency range of what is essentially random noise, together with strong tonal harmonics at 2,000, 4,000, 6,000, and 8,000 cycles.

Tonette†: This instrument may be used either with a long, sustained "open" tone (no stops employed) or with short, sharp blasts of the "open" tone. Its fundamental tone is of the order of 750 cycles and there is one overtone (not strong) at 1,500 cycles.

Details of performance (measured in decibels with a General Radio Sound Pressure Level Meter, read at slow speed with the B network) are as follows:

	12 feet	9 feet	6 feet	3 feet	1 foot
Clacker	64	68	69	70	74
Doorbell	55	56	57	61	72
Tonette	65	67	72	80	89

These details of performance are not meant as calibrations. These are quite different acoustic events, and various details of position and duration inevitably affect intensity readings. Interestingly enough, with a little practice it is possible to repeat regularly with variations not exceeding 2 to 3 decibels.

RESULTS: THE NEWBORN INFANT

Clacker.—All 25 newborn infants, even the one tested at 4 hours of age, responded satisfactorily. In order to obviate the possibility of tactile or skin stimulation as by a draft created by the clacker, it was operated on occasions beside an open door but on the other side of a wall from the baby and if used in the same room, the person operating it interposed his body between the clacker and the baby 6 to 12 feet away.

Doorbell.—One premature infant, aged 1 month, and one premature infant, aged 2½ months, responded. No full-term newborn infants responded.

Tonette.—None of the newborn or premature infants responded even though, as may be seen from the Tables I and II, this was a fairly successful stimulus in older infants.

Group II.—Sixty-four newborn infants have been tested with the clacker in the nursery as part of the routine pediatric neurologic examination done on the second or third day of life. These infants are all babies included in the Cerebral Palsy Study since December, 1958. Good responses have been obtained from 55 babies, 2 have given questionable responses, and 7 have failed to respond at all. Of the 9 who failed to respond adequately, 5 had other neurologic abnormalities. Of the 55 babies giving normal responses, 4 had other neurologic abnormalities. All the babies in the group will be routinely tested again at the age of 4 months.

SUMMARY AND CONCLUSIONS

1. Auditory screening techniques have been developed and tested in three groups of infants: (1) the newborn infant; (2) infants from 3 to 30 weeks of age; (3) older infants, above 30 weeks.

2. The method for the newborn infant involves the use of a "clacker" which produces a stimulus with a

*Manufactured by Liberty Bell Mfg. Co., Minerva, Ohio, catalogue number 2003.

†Manufactured by the Tonette Co., 7373 N. Cicero Ave., Chicago 30, Ill., "C Melody" model.

sharp, short percussive peak of approximately 60 decibels intensity and a frequency range of 8,000 kilocycles. Of 64 infants tested in this manner, as part of a general pediatric neurologic examination, 55 responded with a complete or partial Moro response. Nine failed to respond adequately and of these, 5 showed other neurologic abnormalities. Four of the 64 had normal hearing response but abnormal neurologic findings. This test would appear to be a practical and simple addition to the pediatrician's repertoire.

3. The test method for the infants 3 to 30 weeks of age involves careful watching for a number of possible responses. Of 348 children tested, 25 failed initially to give a satisfactory response. The test takes a moderate amount of skill and experience on the part of the tester. It appears to be a useful diagnostic and research tool but because of the complexity of the responses obtained probably not applicable as a screening device on a general pediatric and public health level.

4. The Ewing test was used for infants over 30 weeks. Of 90 infants between 30 and 52 weeks of age, 4 failed to give a satisfactory response to initial testing. This would appear to be a simple screening device which can be carried out in a clinic setting by nurses with a modicum of special training.

5. The efficacy of various stimuli was tested and a streamlined basic test developed. Where the basic test fails to produce a satisfactory response, additional stimuli are presented.

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TWO SYMPTOMS PATHOGNOMONIC FOR CONGENITAL CENTRAL COMMUNICATION DISORDERS IN CHILDREN

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IDENTIFICATION of the child with a central communication disorder is often unnecessarily delayed; yet early diagnosis may be of great benefit to the child and to his family. Congenital central communication disorders in children may range in severity from total lack of any response to any acoustic stimulus to mild difficulties with use of language as a conceptual tool. The only necessary conditions for a diagnosis of central communications disorder are that the disorder is not attributable to peripheral (end-organ and eighth nerve) auditory dysfunctions or to primary motor-speech dysfunctions. There are no other formal criteria which distinguish central communication disorders from other mental dysfunctions which affect a child's ability to communicate. Such mental dysfunctions include general mental subnormality, various specific or general memory defects, or the numerous types of peculiarities of attention which may affect perceptual functions, more complex behavior, or both. Any one of these conditions itself is capable of causing severe or complete disruption of communicative functions. A child with central nervous system (CNS) dysfunction, bright or dull, and irrespective of the etiology, may have any one or a combination of such conditions. This paper describes two symptoms in children who have no other symptoms except a breakdown in communication skills. They are: (1) failure to make "expected responses" to sound, irrespective of intensity level, and (2) total absence of speech. These two symptoms occur commonly among children with central communication disorders. They are almost always pathognomonic for CNS dysfunction and can be readily recognized by a pediatrician in a routine examination. Popular misconceptions about them are so prevalent among medical and paramedical professional workers that by far the greater part of the cases are first misdiagnosed, and in nearly all cases accurate diagnosis is unnecessarily delayed for many months and often years.

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FAILURE TO RESPOND

Failure to make "expected responses" to sound, irrespective of intensity level, may be called a disturbance in orienting or alerting responses in relation to sound stimuli. It may take the form of consistent absence of response to sound during the child's entire life; on the other hand, it may take the form of responses which are highly variable in

time and in stimulus intensity necessary to elicit a response. The essential characteristic of the pathognomonic symptom is that an examiner intent on eliciting a motor response (as differentiated from an autonomic response) to sound fails to elicit "expected responses."

Use of the term "expected responses" requires an explanation. Although expected responses to auditory stimuli change with the age of the child, an orienting or alerting response to high intensity sounds can be expected of all normal children, regardless of age and including those with severe peripheral impairment of hearing. Even the neonate is expected to respond to a high-intensity sound (banging a hammer on a skillet), although he may not respond to every one of the sequentially presented high-intensity sounds. His response may be a startle reflex, some other motor response, or simply a change of ongoing motor activity. He may start to cry or if he had been crying before the sound, he may stop. However, some demonstrable response can be expected and if followed by a period of silence (say 2 or 3 minutes), a response to subsequent stimuli can be expected again. Similarly, a normal 2- or 3-year-old child engrossed in doll play or other favorite activity can be alerted to a high-intensity sound. If a second and third sound is given within a few seconds of the first, the subsequent sounds may not elicit a motor response. Again, if the first sound is followed by a 2 or 3 minute period of silence, responses can be expected. It was stated above that such responses are expected even in children with severe peripheral impairment of hearing. It should be stressed, of course, that the sound stimulus must be of sufficient intensity. Banging a hammer on a skillet with considerable force at a distance of 6 feet from a child easily produces one hundred decibels of sound pressure, and when a child fails to respond to this stimulus there are grounds for the strongest suspicion of a central involvement.

In children who have never responded to any sound the consistent absence of response is usually detected in the first 6 months of life. However, in a significant number of children with congenital central communication disorders a severe orienting or alerting response disturbance is not discovered by parents or professionals until the child has reached 2 and 3 years of age or later. In these children a lack of speech is often the referring symptom and only complaint, and skin resistance audiometry usually indicates that the auditory dysfunction is not attributable to end-organ or eighth nerve loudness factors. In perhaps one half of the children in whom the orienting or altering response disturbances have not been noticed until 18 months of age or later, there is evidence to suggest that these children had previously made more adequate responses to sound. Failure to continue to make such adequate responses may be due to organic factors present since birth but that become significant only at certain developmental stages. Mark and Hardy[1] offer the hypothesis that failure of the auditory system to serve more complex communicative functions (ability to identify complex sound patterns such as words, for example) may produce disorganized learning processes

which themselves are sufficient to account for the inhibition of previously established auditory functions such as orienting or alerting responses to sounds. Whatever the causes, there can be little doubt that a significant number of children in whom consistent alerting responses to high-intensity sounds cannot be elicited had much more adequate responses at some previous time in their life. It is likely that an alerting or orienting response disturbance emerges gradually; that is, over a period of time there are fewer and fewer consistent responses to even high-intensity sounds. The point at which a family becomes alarmed and seeks professional help is undoubtedly a highly individual matter. One family may be alarmed by a single clear failure to elicit a response; another may be reassured by the occasional responses and be absolutely convinced that the child "hears well" because the occasional responses are quite likely to occur to low- as well as to high-intensity sounds.

Highly variable phenomena, in general, present problems in conceptualization. The inconsistent auditory responses in children with orienting or alerting response disturbances contribute greatly to the prevalent misconceptions about this symptom. The most common misconceptions are that the child fails to respond for psychosocial or emotional reasons or that he has a peripheral hearing loss. The psychologic explanations are usually presented as "he hears when and what he wants to," "he is always too busy to listen," or "this is his way of showing who is boss." Yet it can be stated quite categorically that the overwhelming chances are that failure to elicit expected motor responses to loud sounds by an examiner intent on eliciting such responses is attributable to organic CNS factors. A child who is obviously generally uncooperative may have to be examined at another time; a child who is selectively "uncooperative" to acoustic stimuli only should be examined for a central communication disorder. "Selective hearing" in everyday life situations is a common enough phenomenon so that parental reporting must be screened with some care. However, lack of expected responses by an examiner intent on eliciting a response is most uncommon.

Although peripheral hearing disorders also show variability of functioning, this variability differs in many functionally significant respects from the variability often seen in central communication disorders. An upper respiratory condition may alter the stimulus intensity necessary for eliciting a response by 10 to 50 decibels, but such a condition does not change sensitivity to sound from near normal to the equivalent of a 100 decibel hearing loss. There are other limits to variability which permit children with complex and severely distorting peripheral hearing disorders to hear the complex auditory patterns consistently distorted. Such children learn to associate to the consistently distorted auditory patterns much in the same way that children with normal hearing learn to associate to the undistorted patterns. Their speech often accurately reflects the distortions. They may learn more slowly and fail to make finer discriminations,

but they generally develop considerable auditory verbal comprehension of spoken language.

It is worth noting that responses to extra-auditory sensory cues often hide the pathognomonic symptom of failure to make expected responses to sound, irrespective of intensity level. Many parents cite as typical evidence of frequent responses to loud sounds the fact that "the child turns the radio or television on low when it is too loud." A bright child will easily learn to turn down the volume control on a radio when he becomes aware of low-frequency vibrations through the floor. There are many such cues which help a bright child distinguish between the presence and absence of sound at the better than chance level in an experimental situation and an examiner must be constantly alert to them.

ABSENCE OF SPEECH

Total absence of speech in a child old enough to have speech is also pathognomonic for a central communication disorder. When speech is considered as the production of consistent vocalizations with communicative intent, irrespective of articulation skills, total absence of speech (except in cases of traumatic injury or brain disease) is almost invariably secondary to lack of auditory verbal comprehension of spoken language. Out of thousands of children with central communication disorders seen at the Johns Hopkins Hearing and Speech Center over the last decade, there are fewer than five known exceptions to this general rule.[2]

The most prevalent misconceptions about total absence of speech in an alert child 2 years or older are similar to those misconceptions noted for the first pathognomonic symptom. They are: that the child fails to speak for psychosocial or emotional reasons, that he is just a slow talker, or that he has a peripheral hearing disorder. Many an alert 3 year old who has never made a consistent vocalization to designate a person, object, or situation is said to have "an emotional block." Often parents are assured that "the child will talk when he is ready. He has no need to talk. He makes himself understood and gets what he wants without talking." Parents are frequently given an example of a presumably normal child "who didn't say a word until he was 5." Yet, it can be stated quite categorically that in any intelligent 3 year old who has failed to make some consistent vocalizations with communicative intent, the overwhelming chances are that it is attributable to organic CNS factors totally unrelated to child-rearing practices or psychosocial or emotional factors. A consistent vocalization with communicative intent appears as an item on commonly used developmental schedules (Cattell,[3] Gesell,[4] and Griffiths[5]) at the 9 to 11 months level. All the evidence suggests that variation in the time of emergence of communication skills is similar to the variation in the time of emergence of other mental abilities or motor skills. Thus, there is every justification for strong suspicion of organic dysfunction even in an intelligent 2 year old who makes no consistent vocalizations with communicative intent, provided that the relevant facts about absence of consistent vocalizations and intelligence can be firmly established.

It is not difficult to establish the facts relevant to speech and intelligence and, with care, the probability of making a false positive diagnosis even in a 2 year old can be held to a minimum. If a child has any consistent vocalizations, the mother is usually well aware of them. The pediatrician's chief task is to explain to the parent what is meant by a consistent vocalization. It should be emphasized that such vocalizations do not have to be well articulated; they do not have to resemble English words in any way recognizable to strangers. The vocalizations need only be consistent and have specific meanings. If, after such an explanation, parents still report that there are no consistent vocalizations, the chances are that none in fact exist. Even when the pediatrician considerably overestimates the 2-year-old child's intelligence, the risk and penalty of making a false diagnosis of central communication disorder, based on total absence of speech, are small. If the 2 year old in fact has a mental age of 12 months (that is, an I.Q. of 50), the absence of a consistent vocalization may still be compatible with his general mental subnormality. Such a child may well develop various speech functions at roughly the expected mental ages. If the 2 year old has a mental age of 18 months (that is, an I.Q. of 75), the chances are very good that the child will have some consistent vocalizations unless there are organic factors which retard or prevent the emergence of this skill.

A total absence of speech in an intelligent child 2 years of age or older is sometimes erroneously attributed to peripheral hearing impairment. As previously indicated, where such impairment is in fact the only disability, the 2- to 3-year-old child will almost invariably acquire speech, which often accurately reflects the nature of the hearing loss, at quite a reasonable rate. This speech is usually recognized as characteristic "deaf speech" by professional workers and laymen alike. However, some children with central communication disorders who have no auditory verbal comprehension whatever learn to make a few consistent vocalizations through kinesthetically (rather than auditorily) learned and monitored speech. Children with kinesthetically learned vocalizations usually can be distinguished from children with peripheral hearing disorders by their inability to understand any spoken language, discriminate among grossly different sounds, or respond to the presence or absence of a sound. In addition to a difference in voice quality such children rarely learn to make more than 3 or 4 such kinesthetically learned consistent vocalizations between the ages of 2 and 3 years, unless of course there has been some special teaching effort. If a child has no more than 4 or 5 consistent vocalizations between the ages of 2 and 3 and if he cannot discriminate between or identify grossly different sounds, the chances are still very good that he has a central communication disorder. If, however, he can understand some language through hearing alone and has more than 4 or 5 consistent vocalizations, the differential diagnosis between a peripheral hearing versus central communication disorder cannot be readily made by common office procedures and requires examination by specialists.

PROGNOSIS

The two symptoms and other phenomena described are common among children with central communication disorders. They are probably more prevalent in children with other symptoms of mental dysfunction than they are in children in whom such symptoms are totally absent. All children with suspected CNS dysfunction should have a careful expert evaluation at the earliest possible time. One reason for describing symptoms in the "bright" child who has no other alerting signs is that this child probably has the best prognosis for improvement in those general communication abilities which are not dependent upon auditory verbal comprehension and speech. Yet, he is least likely to get an early referral to specialists.

Little is known about prognosis for major symptom improvement in children with central communication disorders. There are no control studies to test the efficacy of educational techniques or therapies in improving specific symptoms. There are no data on spontaneous remission rates for specific symptoms. The history of the specific symptom is probably the best single, though highly fallible, index for predicting the future course of the symptom. Symptom improvement in central communication disorders should not be confused with symptom improvement in peripheral hearing disorders. In the latter there is good evidence that early detection and correction by a hearing aid and special training techniques serve to minimize the effect of the disorder on the learning of more complex language functions.

Lack of scientific data regarding the course of specific symptoms in no way obviates the need for early and accurate diagnosis. Correct evaluation of a child is basic to planning a program designed to elicit optimal communicative as well as general functioning. Early and accurate diagnosis also saves the child and family the tensions that come with attempting to fit observed behavior into inadequate models. When the model is one involving psychosocial causes for a condition in a child, parents often feel responsible and anxiety and tension are likely to be great.

SUMMARY

This paper suggests that (1) failure to make expected responses to sound irrespective of intensity level and (2) total absence of consistent vocalizations with communicative intent in a child old enough to have speech are symptoms pathognomonic for central communication disorders in children. Popular misconceptions about these symptoms are presented, and methods useful in identifying the symptoms during a routine pediatric examination are discussed. The need for early and accurate diagnosis is noted.

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PROBLEMS OF ADOLESCENCE

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EVERY day of man's life constitutes a transition between yesterday and tomorrow, converging in the intrinsic values of the moment. The past and present continue to shape and modify directions and goals, mostly through quiet evolution, sometimes in lively spurts. One of the liveliest spurts occurs during adolescence, at a time when a person is no longer a child and not yet an adult.[1, 2]

Many incisive changes take place in that period. Body growth, in a remarkable upward surge, attains its maximum for the individual. The physique assumes its characteristic configuration. Sexual development reaches procreative capacity. There is striving for emancipation from sheltered existence, a trend toward increasing self-dependence in thought and action. The sphere of interest and participation expands from the confines of home, neighborhood, and school to the community at large. The choice of vocation, until then a playfully considered matter, becomes a real issue. Current standards and precepts are submitted to criticism not as yet leavened by the tests of experience. The established order is challenged boldly and then, after some struggle, appropriated gradually with more or less reservation.

Adolescence, in our culture, is a great translator. It translates the language of parental direction, attitudes, and behavior into an individualized idiom. This is much less evident in primitive cultures. One might go so far as to say that in primitive societies there is no conceptual equivalent for that which we call adolescence. Childhood ceases abruptly when, through a set of elaborate rites, it is transported into full-fledged adulthood. Tribal ritualism, rather than personal spontaneity, determines status and function. In our social structure a child is given several years in which he is to find his way from a more or less manipulated and regimented existence to the acquisition of initiative in a loosely competitive environment in which the taboos are blurred, the semantics are equivocal, and the variety of occupational, political, and theologic choices offers opportunities for perplexities. The adolescent translator's dictionary is full of confusing synonyms and antonyms.

A combination of inner soundness, wholesome parent-child relationship, and guidance from understanding adults helps most adolescents to emerge safely from the groping and floundering which precede maturing stabilization. Overreaching or falling short of the target can be attenuated by bringing aimer, aim, and target into reasonably comfortable harmony. However, the great variety of backgrounds and personalities is fraught with possibilities of less easily smoothed conflicts and clashes. Developmental shortcomings, early exposure to pathogenic

parental attitudes, and burdensome life situations may work singly or together to intensify perplexities and twist emotions and performances. Hurdles taken by others with sufficient skill and economy of effort may come to loom as conspicuous barriers, circumvented inefficiently, avoided shrinkingly, or allowed to inflict serious damage in clumsy and incongruous attempts to surmount them.

The normal adolescent has his puzzlements and preoccupations; trouble starts when the puzzlements and preoccupations threaten to have the adolescent. It is then that the tasks of accepting one's identity, fitting oneself to things and people as they are, and establishing and maintaining give-and-take relationships come to a crucial test. Sensitiveness about physical appearance and functioning may become a predominant feature, with excessive self-observation and logically as well as empirically inadmissible causal associations. Prevailing superstitions and pamphlet lore, sometimes echoed even by physicians, make masturbation a focus of worry and dread. The search for social and cosmic orientation confronts the fledgling with ill-defined generalizations which he can brush aside without much ado, explore in pseudo-philosophic ruminations and debates, eventually sift for practical usage, or confuse to a degree out of proportion with utility and reality. Ambitions may fall in line with expectations and serve as guides for consistent planning, lag behind expectations through want of ability, purpose, initiative, or self-confidence, or shoot far beyond the attainable mark in fantasies but little related to actualities or in jerky, misdirected, prematurely spent thrusts.

Many young people, well prepared biologically and educationally, go through the second decade of life without essential disturbance. Some get entangled temporarily but emerge steadied, pulling themselves up on their own bootstraps or, if need be, holding on to a guiding hand. Others may become so thoroughly engulfed in all-embracing moods, ensnared in abstractions, trapped in blind alleys of mystic preoccupations, drawn away from realities, or rudely tossed into realities that they are in serious danger of getting lost in their efforts to find themselves and their place in the scheme of things. These are the persons whose road to adult socialization often leads over bumpy detours from which the main highway may or may not be regained. The detours, more or less profound deviations of feeling, thinking, and acting, may take the shape of overpowering anxieties, obsessions and compulsions, hysterical reactions, delinquent behavior, alcohol and drug addiction, depressions and elations, and disorders of the schizophrenic variety.

There was a time in the not-too-distant past when the inception of a psychiatric problem was regarded as coincident with the appearance of observable symptoms. This is fully justified from the point of view of descriptive clinical pathology. It is a practice common to all branches of medicine; the "onset" of an illness is dated from the time of the first subjective complaint, objective manifestation, or both. Medicine, nevertheless, has come to appreciate the significance of incubation periods, insidious bacterial invasions which take some time before they announce themselves symptomatically, tumor formations which

betray their presence only after a period of hidden expansion.

These realizations are a help in the understanding of analogous developments in the field of medical psychology. The onset of behavior symptoms is not the onset of the problem; it is the point at which the problem has broken through to the surface, has become manifest through its externalization, through the annoying or frightening form which it assumes, through the nuisance value which it represents. The onset of the clinical picture is, in effect, an outcome, at least a temporary outcome, rather than something which comes as a bolt from an untroubled sky. It rouses curiosity about the nature of the pathogenic agent which, instead of being the cumulative effect of a toxin or the spreading encroachment of a tumor, is a combination of psychodynamic forces closing in on the person. It is, when it occurs in adolescence, a set of harrowing experiences interfering with composure and smoothness of functioning at an age when parental cushioning or parental intimidation can no longer be tolerated as a substitute for desperately needed opportunity for self-expression.

It may be said without exaggeration that there is no major psychiatric problem in adolescence which does not have its origins in earlier life experiences. The character of these experiences not only forebodes the probability of the occurrence of personality difficulties but also predetermines the patterns in which the difficulties will express themselves if the pathogenic constellations persist.

What are these pathogenic constellations?

Every human being, starting out in life as a biologically helpless creature, grows into maturity to the extent to which he can attain a fully differentiated identity. Emotional growth is essentially a successful struggle for identity. It results in a progressive development of self-dependence and self-reliance, a gradual emancipation from an initially receptive and submissive subordination to direction by adults. The degree and form of the emancipation are governed by cultural pressures for an irreducible minimum of conformity, identification with the parents and other persons important in the child's life, and parental attitudes expressed in behavior which may encourage or suppress spontaneity.

Some people find it difficult to see how experiences in infancy and early childhood can so affect an individual's emotional equilibrium that they could burst out in delinquent, neurotic, or psychotic flames in adolescence when no noticeable smoldering seems to have preceded. The connection would be more easily comprehended medically if two things could be demonstrated convincingly: first, that noxious attitudes can play as much havoc with personality formation as organic infringement on brain structure, and second, that an interval of apparent conformity does not always signify emotional health in a child subjected to noxious attitudes.

The first demonstration, which is one of the major contributions made to psychiatry in the 1940's, has been offered by Goldfarb[3] and verified by others. Goldfarb studied two groups of adolescents. The one consisted of persons who in their first 3 years of life had been cared for in orphanages where they had all the prescribed nutritional and hygienic attention but

were deprived of personal stimulation and affection. The control group was made up of boys and girls of the same social strata who were reared in foster homes from the beginning. The majority of the orphanage group, even though they were later transferred to foster homes, were so seriously injured by the early emotional deprivation that their adolescent behavior, their psychometric and Rorschach responses showed marked resemblance to the essential features of postencephalitic reactions. This was not at all true of the members of the control group.

The second point, that of apparent normalcy prior to the clinical onset of disturbing symptoms in adolescence, also has an analogy in organic pathology. Juvenile paresis may not come to the fore until the patient is well along in the second decade of life. He may have done reasonably well up to that time and presented no problem, even though the spirochetes had been active from the beginning and worked busily toward disaster.

The apparent normalcy of an adolescent headed for psychiatric upheaval is largely a matter of evaluation, as is the scenically beautiful calm of a seemingly quiescent volcano a short time before its eruption. Wickman's pioneering book, published almost 20 years ago, has pointed out flaws in the evaluation of children's behavior, flaws which are still prevalent. Wickman[4] asked teachers and psychiatrists to rate children's behavior items from the point of view of their seriousness. Teachers stressed the pathologic importance of self-assertive behavior which offended their sense of decency, dignity, or authority. They were not impressed by meekness, submissiveness, obsessive meticulousnes, or indications of withdrawal which were regarded by the mental hygienists as far more ominous than aggressive conduct. Laymen generally tend to judge children's behavior on the basis of its nuisance value. Hence, the deceptive impression often persists that breakdowns in adolescence may arise without warning; the warnings expressed through a child's anxious bid for acceptance and approval, through his striving for perfection, through his timidly unquestioning obedience, far from being recognized as storm signals, are mistaken for wholesome conformity by autocratic adults. It is often pathetic to listen to the puzzled account of parents of adolescents brought with all the evidences of a seemingly sudden schizophrenic catastrophe. How, they ask uncomprehendingly, could this have happened to their child who had always been so excessively good, polite, obliging, industrious, a prodigious reader, the pride of the teachers who have always given him "excellent" marks in conduct—in short, a model child?

There may be a degree of truth in the saying that the tree will incline as the twig is bent. However, many adolscent problems arise from an overbending of the twig to the point of breaking.

Quite a bit, of course, will depend on the elasticity of the twig itself, something that has been referred to variously as constitution, inherent personality structure, ancestrally predetermined tendencies, basic ego strength, and the like. The cortical cells, the basal ganglia, the glands of internal secretion, the types of body configuration, and other factors have been dragged in for an explanation of the differences of individual responses

to situational perplexities. It has been customary to fall back on the word "puberty" as a sort of bugaboo supposed in some diffuse and mysterious way to set off emotional fireworks because of its very existence. There is an analogous trend in the lay public to ascribe neurotic problems of middle-aged women indiscriminately to the word "menopause" or, as the vernacular has it, "change of life."

There are different kinds of twigs, to be sure. Nevertheless, all of them have a chance to become sturdy trees provided that they grow in good soil, are not afflicted with crippling diseases, and have the nutrition and sunshine they need. Let someone keep pulling constantly at a twig, flood the soil incessantly, try to insist that a young forsythia become an oak or an oak become a forsythia, wrap it in protective layers of cotton, overheat or overfreeze it, try to change the direction of the sprouting leaves—the result may not be a very healthy tree or bush. The human twig needs a good home, physical care, and adequate nutrition. Also he needs an opportunity to unfold his potentialities, the potentialities which are uniquely his, with a minimum of the kind of agitation which frustrates naturalness and spontaneity.

Close observation of parent-child relationship in our culture leads to the conclusion that parents much too often bend and overbend, overheat and overfreeze their children to serve their own complex emotional needs.

Children can be comfortably warm, swelter, or freeze in the emotional climate maintained by parental attitudes. The homes in which they grow up can be pleasantly air-conditioned places, ovens, or refrigerators. Children can be objects of enjoyment, recipients of anxious overprotection, organs of parental hypochondriasis, screens on which hostilities are projected in a cold war or in the form of unmitigated atrocities, often the fields of battle on which the fathers and mothers, sometimes with varying strategies, test the tactics of their campaigns.

The statement may be premature, because not as yet backed by statistical material, but is nevertheless justified on empirical grounds, that children reared in a comfortable emotional climate have the best chances to become well-balanced adolescents and adults. Many of the others may begin to show the effects in the years of childhood, carrying them over into adolescence unless something happens to improve the climate. However, there are many who do not externalize their conflicts until circumstances force them to translate the established domestic relationship patterns into terms of relationships with the world at large. If you wish to translate, you can do so only when you have had some experience with the language used in the translation. These adolescents have been kept from learning the language spoken by the majority of free people. They are familiar only with one limited type of communication, that which has been impressed on them and pressed into them for years. It is a language which says in effect: "You better remain under the protection of parental regimentation because you have never been taught how to rely on your own initiative." Or it says: "You know exactly what we expect of you and you owe it to us to live up to these expectations regardless of how you feel about them; in fact, you have

no right to have any feelings of which we do not approve." Or it says: "Your recent departures from one hundred per cent compliance are signs of treason. Your arrival last night half an hour past the allotted time, your failure to hang up the sweater, the opinion you expressed the other day about grandfather or the political candidate, the teacher's report about your saucy remark in the classroom, all these are signs of depravity about which you should feel guilty and duly apologetic."

There are some adolescents, as there are some nations, who have no need for a drastic act of emancipation. They are the boys and girls born and reared in the relative freedom of a domestic democracy which is a combination of mutual understanding, tolerance, compromises, and an invitation to conformity rather than an enforcement of surrender.

There are other adolescents, as there are other nations, who, having experienced autocracy, develop sufficient maturity to strain at the shackles and to throw them off with the aid of relatives, teachers, friends, or physicians.

Again there are adolescents, as there are nations, who never achieve the equivalent of a Fourth of July. Revolt, nurtured by the accumulation of unbounded and unredeemable hostility, has led one nation to the mass use of the guillotine; it has started some adolescents on the road to delinquency and crime, with a transfer of vindictive resentment from the parental oppressors to everyone in the path of their retaliatory aggression. Submission, held up for centuries as a virtue, has made of another nation, famed for its poets and philosophers, the complying tool of one of history's most hideous despots. Some adolescents have become so inured to unconditional surrender that they are unable to cope with the ineseapable need for meeting the challenge of communal adjustment. The intrenched habit of seeking approval through excessive meticulousness and the search for perfection in an imperfect world may drive the frustrated seeker to preoccupation with obsessive symbols which place perpetual obstacles between the patient and ordinary routine functioning. On rare occasions, the overwhelming impact of demands on the adolescent's emotional resourcefulness may result in the conversion of the conflicts into paralyzing, blinding, or deafening hysterical phenomena.

Adolescence is the age par excellence at which the temptation to give up, to get away from it all, to substitute unchecked fantasy for frightening reality, to regress to the sheltering comforts of infantile passivity can become so alluringly irresistible as to make schizophrenic capitulation attractive. The textbooks of psychiatry state that schizophrenia often begins in adolescence. This is correct only in the sense in which it would be correct to say that a war begins on the day when the shooting starts. Nevertheless, the shooting has been preceded by rivalries and hostilities of long standing. The schizophrenic manifestations are a culmination, a radical withdrawal from realities which have for a long time played havoc with the patient's emotional equilibrium. Schizophrenia is a drastic retreat to isolationism. It is a case of "cold feet" resorted to when there are no other resources left, when goodness, dependence, quickly crushed abortive attempts at rebellion are no longer

serviceable. It is a refusal to make further emotional investments after so many previous investments have yielded no returns. It is an escape from the unpleasantness of further rebuffs.

Once the storm has broken and a problem has become manifest, the psychiatrist is the only person prepared to struggle with it. The struggle is easy or difficult, depending on the patient's and the parents' felt need for help, the virulence of noxious attitudes, the degree to which the adolescent's emotional distress has intrenched itself, and last but not least the therapist's own personality and therapeutic skill. This is equally true of all other medical concerns. In psychiatry, as in all other fields of medicine, there are benign and malignant conditions. Progress in medicine is often measured rightly by the extent to which potential malignancies can be recognized at the earliest possible time and so treated that ominous developments are nipped in the bud.

While the treatment of psychiatric problems which have arisen should be left in the hands of psychiatrists, pediatricians and general practitioners are in the best strategic position to recognize and alleviate those pathogenic situations which, if unrecognized and not alleviated, work toward discomfort, maladjustment, and possible malignancy. The pediatrician is the family's natural adviser while the child passes through the years from infancy to and through adolescence.

The pediatrician's role as a mental hygienist can do much to prevent or nip in the bud behavior problems of small children and at the same time to prepare conditions best suited to guarantee a reasonably wholesome adolescence and adulthood. His principal function may be compared loosely to that of a thermostat, a regulator of the emotional climate in which the child is brought up. However, while a thermostat functions automatically, the pediatrician's function requires a great deal of personal sensitiveness, orientation, and experience. He will then sense the excessive heat of overprotection, the type of mothering which smothers, the chill of parental rejection peering through a thick coat of coercive perfectionism.

The modern pediatrician has put these concepts and practices to a test. He has found them empirically sound. He is learning to appreciate the etiologic and personality-shaping significance of parental attitudes. He knows that noxious attitudes cannot be modified merely by telling a mother that she should not overprotect, be overanxious, coerce, and hold on to an umbilical cord which has not been severed psychologically. The parental carriers of noxious attitudes are not villains who wish to drive their children into confusion, dependence, and emotional agony. They are troubled people with perplexities and anxieties of their own. Their psychotoxic condition cannot be altered by admonitions and may be aggravated by condemnation. However, the toxicity can be reduced by understanding, by giving the parents a chance to come out with their feelings, by rendering them the kind of support which, coming from an accepted and respected authoritative source, reassures, mitigates anxieties, removes the emotional basis from factually unwarranted solicitudes and agitations.

Thus, the pediatric thermostat can help, wherever necessary, to maintain

or create a comfortable emotional climate for the child who can then find his identity without struggling unsuccessfully to throw off the blankets of smothering overprotection or shivering in the chill of parental rejection. There is no better and no alternative prophylaxis against psychiatric problems of childhood or adolescence. It is a common experience that a person who comes out of a comfortably warm house can stand the vicissitudes of the outside weather much better than one who comes out of an overheated or a chilled house.

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Comments on Current Literature

TRACHOMA AND INCLUSION BLENNORRHEA

THE infectious agents of trachoma and inclusion blennorrhea occupy an unusual position in the viral group of microorganisms. While the etiology of trachoma was investigated very early, notably by Julianelle and Harrison,[1] laboratory research in trachoma has not been as productive as that in other virus diseases. As Thygeson[2] points out in his recent review, "The Present Status of Laboratory Research in Trachoma," the chief reasons for this lag have been the lack of a suitable experimental animal and the failure of the disease to invoke a serologic response of any strength. Likewise, failure of the virus to grow readily in tissue culture has hindered the investigation of its biological properties, and Thygeson emphasizes that for a satisfactory study of this agent higher primates or human volunteers are needed. In this authoritative review published in the *Bulletin of the World Health Organization* in 1958, pertinent facts concerning trachoma are summarized and the inclusion blennorrhea syndrome is discussed incidentally.

In the case of both of these agents infection of the eye can be detected by the presence in the conjunctival exudate or scrapings of inclusion-bearing epithelial cells which can be seen readily in preparations stained by appropriate techniques. The characteristic cytoplasmic inclusion body has been shown to be composed of elementary bodies in a carbohydrate matrix. These agents resemble viruses in their filtrability, their cytotropism, and in their tendency to produce inclusion bodies; they resemble the rickettsiae in their staining characteristics, blue staining with Castaneda's, and red staining with Macchiavello's stain. More important from the clinical point of view is their susceptibility to the sulfonamide drugs and broad-spectrum antibiotics. Tentatively, these infectious agents have been classified in the psittacosis-lymphogranuloma group of obligate intracellular parasites, intermediate between the rickettsiae and the smaller viruses. Pending revision, the generic name Chlamydozoon has been suggested for this group of agents, that of trachoma to be designated as *Chlamydozoon trachomatis*, and that of inclusion conjunctivitis as *Chlamydozoon oculogenitale*.

Until recently, attempts to cultivate the virus of trachoma in the embryonated hen's egg had not met with any real success.[3] In recent publications, however, reports appear which indicate that the viruses of trachoma and of inclusion blennorrhea can be isolated and propagated in the developing hen's egg, especially by yolk-sac inoculation. One of the May issues of *Lancet*, in 1958, carried such a report by Collier and Sowa.[4] Confirming earlier results by a group of workers[5] in China (1957), these investigators were able to isolate and propagate a strain of trachoma virus originating from conjunctival swabs of trachoma patients in Gambia, West Africa. In this

instance verification was accomplished by transfer of egg-passage material to a human volunteer. Characteristic inclusion-bearing cells were seen in the resulting exudate. From this volunteer the virus was reisolated by yolk-sac inoculation with typical inclusion-bearing cells occurring, as before, in the epithelium of the yolk sac. Further study of these experimentally propagated strains of trachoma virus was reported by Collier, Duke-Elder, and Jones[6] in the *British Journal of Ophthalmology* for December, 1958. In the same issue Smith[7] of the Evans Biological Institute, Cheshire, described an accidental laboratory infection with trachoma virus in which a laboratory worker inadvertently had inoculated his own eyes with virus-containing material. Five days later typical symptoms appeared; the incubation period was short and the manifestations severe, probably because of a relatively high concentration of virus in the material being handled. Rather slow but complete recovery followed the use of broad-spectrum antibiotic therapy. The agent was reisolated readily by means of yolk-sac inoculation. On the basis of these studies it seems likely that the virus of trachoma can be cultivated successfully in embryonate eggs using the yolk-sac technique. Transfer of this material to human volunteers, with resulting clinical disease of characteristic nature, and subsequent transfer back to the developing hen's egg with continued infectivity would seem to indicate that the virus does multiply in the yolk sac and is readily adaptable to either host. In this respect the virus resembles the rickettsiae which reproduce readily in the yolk sac of the embryonate egg.

A more recent publication by some of these same authors, Jones, Collier, and Smith,[8] appears in the May 2, 1959, issue of *Lancet*, and concerns the "Isolation of Virus From Inclusion Blennorrhoea." The clinical manifestations resulting from this infectious agent include an abacterial form of ophthalmia neonatorum, a nonbacterial urethritis in the male, and a nonbacterial infection of the urogenital tract of the female. Many studies have shown that characteristic cytoplasmic inclusion bodies can be demonstrated in the conjunctival cells of the infected newborn infant, in the epithelium of the urogenital tract of the mother, and in cells from the urethras of male patients. These inclusions are very similar to those seen in trachoma and like the trachoma virus the agent of inclusion blennorrhea has long resisted attempts at culture. Swimming-pool conjunctivitis in older children and adults, a problem of some importance before chlorination was introduced, is thought to result from infection with inclusion blennorrhea virus due to water contamination. This type of conjunctivitis is still being reported in recreational communities near small lakes and among persons frequenting unchlorinated pools.

The recent publication in *Lancet*[8] records the isolation in two independent laboratories of a virus strain from the cervix of a mother whose newborn infant developed inclusion blennorrhea and from the eyes of another infant with the disease. These two family groups were studied intensively. In the first family the mother, a young primipara, had been treated locally because of a moderate vaginal discharge which had become troublesome toward the end of her pregnancy. Conjunctivitis of the left eye was apparent in her newborn infant by the third day of life. At 13 days of age, the baby was admitted to the eye hospital because of a moderately severe mucopurulent conjunctivitis. On admission the conjunctiva was red, edematous, and diffusely infiltrated; the cornea appeared normal. Examination of the mother of this infant revealed that the vaginal discharge was still

present 23 days post partum. Cervical smears showed the presence of many degenerating epithelial cells, together with an inflammatory exudate of polymorphonuclear leukocytes, lymphocytes, some plasma cells, and some macrophages. On two occasions cervical scrapings were collected. In both smears and scrapings some of the epithelial cells showed characteristic intracytoplasmic inclusion bodies. These cervical scrapings were sent for study to two separate research institutions. Both laboratories reported the isolation by means of yolk-sac inoculation of an agent similar to the trachoma virus. In the second family the mother gave no history of leukorrhea before or during pregnancy. Cervical scrapings taken 22 days after delivery were not very satisfactory because of the patulous cervix; however, no inclusion-bearing epithelial cells were observed. This infant was admitted to the eye hospital at 14 days of age because of a bilateral mucopurulent conjunctivitis, recurrent since the third day of life. The condition responded slowly to broad-spectrum antibiotics, the cornea remaining clear. Bacterial cultures of the conjunctival exudate yielded no growth. Inclusion-bearing cells were observed in the conjunctival scrapings, and samples of these scrapings were forwarded to both research laboratories. Again, isolation of the infectious agent was accomplished by yolk-sac inoculation. After a number of egg passages one of these strains of virus was transferred by swab to the right eye of each of 4 young baboons (*Papio papio*). The left eye in each case was treated with a similar virus suspension that had been heated at 100° C. for 15 minutes. Four days later there was no significant change in the appearance of "control" left eyes, and none in the cytology of the conjunctival scrapings made from them. By contrast, the "conjunctivae of the right eyes were congested, oedematous, and exuding mucopus." Conjunctival scrapings and inflammatory exudate contained degenerating epithelial cells showing typical intracytoplasmic inclusion bodies, with large numbers of elementary bodies lying free in between the cells.

This newly isolated strain of inclusion blennorrhea virus was compared in detail with two strains of trachoma virus and found to be very similar in morphology and staining reactions. "Electron microscopy revealed elementary bodies identical in appearance with those of trachoma . . . and with other members of the psittacosis-lympogranuloma group."[5] Like trachoma, this strain of virus was shown to contain the complement-fixing antigen of the psittacosis-lymphogranuloma group of infectious agents. Antigens from the trachoma strains and from the strains of inclusion blennorrhea virus were tested against the serum of a case of lymphogranuloma. On the basis of these recent studies Jones, Collier, and Smith conclude that in morphology and in staining reactions the viruses of trachoma and inclusion blennorrhea resemble other members of the psittacosis-lymphogranuloma group with which they share a complement-fixing group antigen. From the clinical point of view these authors emphasize the importance of early diagnosis, especially in conjunctival infection with these viruses, and stress their susceptibility to the sulfonamides and broad-spectrum antibiotics.

RUSSELL J. BLATTNER

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Erratum

In the article entitled "On the Protein Allowances for Young Infants," by Harry H. Gordon, M.D., and Angelita F. Ganzon, M.D., which appeared in the April issue of the JOURNAL, in Table II, p. 510, formula C should read carbohydrate 15 Gm. instead of 20 Gm. if the formula is to contain 0.67 cal. per cubic centimeter.

News and Notes

The following pediatricians were certified by the **American Board of Pediatrics, Inc.**, on June 19, 20, and 21, 1959, in San Francisco, California.

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The following passed their examination in New Orleans, Louisiana, in March, 1959, but were not eligible to receive certificates until July 1, 1959.

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Dr. Eleonora Posadas Dacanay, c/o Mrs. Encarnacion P. Dacanay, 12-B R. Soliman, Proj. 4, Quirino District, Quezon City, Philippines.
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The 1959-1960 **Institutes for Physicians and Nurses in the Care of Premature Infants** at the New York Hospital-Cornell Medical Center are scheduled to start on the following dates: Sept. 21, 1959, Nov. 2, 1959, Jan. 4, 1960, Feb. 8, 1960, and May 9, 1960. The attendance at each Institute is limited to six physician-nurse teams. The program for physicians is of 2 weeks' duration and that for nurses of 4 weeks' duration. Participants pay no tuition fee and stipends are provided to help cover expenses during attendance at the Institute.

For additional information, write Box 143, Institute in the Care of Premature Infants, The New York Hospital, 525 East 68th St., New York 21, N. Y.

The **Louisiana Chapter of the American Academy of Pediatrics** will present a fall scientific session under the sponsorship of Lederle Laboratories on Friday, Sept. 18, 1959, at the Capitol House, Baton Rouge. The theme of the seminar will be *Malignancies in Childhood.* There will be no registration fees.

For any additional information, write Dr. F. M. Harris, Jr., Secretary-Treasurer, 1401 N. Foster Dr., Baton Rouge 6, La.

The New York Academy of Medicine will hold its Third Annual Postgraduate Week October 5 to 9, 1959. Entitled "Research Contributions to Clinical Practice," the program will include daily evening lectures, daily afternoon panel meetings, and a scientific exhibit.

Nonfellows of the Academy must register but there is no fee. Inquiries should be addressed to Secretary, Postgraduate Week, The New York Academy of Medicine, 2 East 103 St., New York 29, N. Y.

Applications for Research Traineeships to begin before Sept. 1, 1960, are now being accepted by the Research Training Program of the Department of Pediatrics, **University of Washington, School of Medicine**, Seattle. The program is limited to graduates with a degree of medicine. Traineeships are for a minimum of 2 years. Stipends conform to the levels announced by the National Institutes of Health for postdoctorate fellows.

For additional information and application forms, write to: Dr. Bruce Mackler, Department of Pediatrics, University of Washington, School of Medicine, Seattle 5, Wash.

The Awards Committee for the **Mead Johnson Program for Pediatric Research** of the American Academy of Pediatrics announces the availability of funds for the coming year to provide financial aid to pediatric research workers. The maximum amount for any one grant in any year is $3,500. Grant applicants should be young pediatricians who are not more than 10 years beyond the completion of their residency. Deadline for receipt of grant applications for July, 1960, will be Jan. 15, 1960.

Interested persons should write to: American Academy of Pediatrics, 1801 Himan Ave., Evanston, Ill.

The **Sister Elizabeth Kenny Foundation** announces continuation of its program of postdoctoral scholarships to promote work in the field of neuromuscular diseases. Each grant will provide a stipend for a 5 year period at the rate of $5,000 to $7,000 a year, depending upon the scholar's qualifications.

Inquiries regarding details of the program should be addressed to: Dr. E. J. Huenekens, Medical Director, Sister Elizabeth Kenny Foundation, Inc., 2400 Foshay Tower, Minneapolis 2, Minn.

On Sept. 22 to 24, 1959, the **University of Minnesota** will present a continuation course in Pediatrics for Pediatricians at the Center for Continuation Study on the University campus.

The visiting faculty will include Dr. Carl A. Genzell, Professor of Obstetrics and Gynecology, Dr. Konung Gustaf V. Forskningsinstitut, Stockholm, Sweden, Dr. L. Emmett Holt, Jr., Dr. Leo Kanner, Dr. Jack Metcoff, and Dr. Irvine McQuarrie. Dr. Holt will also present the Fifth Irvine McQuarrie Lecture at 8:15 P.M., Wednesday, Sept. 23. The course will be presented under the direction of Dr. John A. Anderson, Professor and Head of the Department of Pediatrics.

Lodging and meal accommodations are available at the Center for Continuation Study.

Applications for a Residency in Pediatric Oncology from July 1, 1960, to June 30, 1961, at Memorial Center for Cancer and Allied Diseases, New York, will be received up to Nov. 1, 1959. This residency is approved for partial credit by the American Board of Pediatrics and is limited to the neoplastic diseases of children. Salary is $3,600 annually plus maintenance. For application forms and information, write to Director, Pediatric Service, Memorial Center for Cancer and Allied Diseases, 444 East 68th St., New York 21, N. Y.

Dr. Edith M. Lincoln, who started the chest clinic of the Children's Medical Service, Bellevue Hospital, New York City, in 1922, and served as its chief until her retirement in 1956, was awarded the Trudeau Medal of the National Tuberculosis Association May 26 for her work in the drug treatment of tuberculosis in children.

Dr. Lincoln is the fourth woman to receive the award, the former ones being Dr. Florence R. Sabin, Dr. Florence B. Seibert, and Miss Emily Bissell.

Dr. Horst Bickle of Marburg Lahn, Germany, will give the Third Annual Grover Powers Lecture at the Yale School of Medicine, Oct. 13, at 5:00 P.M.